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Orphan G Protein-coupled Receptors in Post-Genome Era

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In **'Nature'**, Dixon et al. reported the first cloned mammalian G-protein coupled receptor sequence (1). The DNA sequence from a hamster encodes the β_2-aderenergic receptor. In the same year, 1986, Kubo et al. published the muscarinic acetylcholine receptor sequence (M_1) from a rat in the same journal (2). Both groups purified the receptor proteins and identified the DNA sequences (1,2). These novel findings opened a new era of pharmacology, a period I would call the 'receptor-hunting period'. Lots of G-protein coupled receptors (GPCR) for known ligands like histamine, serotonin, prostaglandin and even morphine have been cloned by PCR (polymerase chain reaction), low stringency hybridization, and expression cloning techniques (3). Now, the Human Genome Project is completed and all the candidate genes are available to be studied (4,5). Almost 350 GPCRs are considered as functional receptors in human beings excluding olfactory receptors (6). Among the genes, about 200 GPCRs are matched with their own ligands. However, the rest of the GPCRs are classified as 'orphan receptors', because their natural ligands are not yet known (6). Considering that half or more than half of the drugs on the current market are acting on the GPCRs positively (agonists) or negatively (antagonists), the orphan receptor project is a fascinating 'goldmine' to the pharmaceutical companies and academia scientists. In my speech, I'd like to introduce the techniques useful for the orphan receptor research with several successful examples and prospect the future of the orphan receptor research.

I. Characters of GPCR

Each GPCR binds specifically to its own ligand and signals via G proteins to the inside of cells. Receptors have seven transmembrane domains. Each receptor has its own selectivity to G proteins, like β-adrenoceptors to G_s and α_2-adrenoceptors to G_i. It is possible to presume a candidate ligand from amino acid identity of an orphan receptor to ligand-known receptor(s). High homology of amino acid sequences with known receptors sometimes gives an idea, if the identity is high enough (like $\geq$ 45%). Sphingosine 1-phosphate receptor (Edg-1)

has a high identity to other members of Edg orphan receptor subfamily (7). Edg-3, Edg-5, Edg-6 and Edg-8 have been characterized as sphingosine 1-phosphate (S1P) receptors serially (7,8). But in many cases, the identity of orphans to ligand-known receptors are too low ($\leq$ 35%) to convince the ligand.

II. Expression cloning techniques with known ligands

When you have a ligand and search receptor(s) for the ligand, the useful technique that has been applied is 'expression cloning' (3). Lots of receptors for the known ligands were found with this technique. Discovery of $CysLT_1$ leukotriene D_4 receptor is an example of successful use of this technique (9). Lynch et al. used frog oocytes (*Xenopus laevis*) for receptor expression, because oocytes are devoid of LTD_4 responses like responses to other GPCR ligands (Oocytes have been a popular tool for GPCR research). Expression of a hundred of orphan receptor mRNAs to each oocyte and challenging each oocyte with LTD_4 led successfully to the identification of an orphan receptor (HG55, the former orphan name) as $CysLT_1$ LTD_4 receptor (9).

III. Reverse pharmacology with orphan receptors

As described in the introduction, we have about 150 orphan receptors but are out of the ligand pool for the orphan receptors. From the late 20th century, pharmacologists started to search natural ligands with cloned orphan receptors. We call this strategy 'reverse pharmacology', because it starts from receptor DNAs in contrast to the traditional pharmacology, which started from drug molecules or disease studies and resulted in receptor molecule discovery at the end.

A strategy is high-throughput screening on the orphan receptor library with a massive library of candidate chemicals. Out of over 700 chemicals, UDP-glucose was found to be a ligand for the orphan receptor, KIAA0001 (10).

IV. Prospective

From the late 20th century, many pharmaceutical companies launched orphan GPCR projects and made great contributions to the discovery of important natural ligands. All these findings are now followed by intensive studies on the physiological and pathological meanings of the novel ligands. Table 1 summarizes some orphan receptors, their ligands, and known functions (7,9,11-20). Still a lot of orphan receptors are waiting for the discovery of their natural ligands by scientists. For drug discovery, however, we have to continue basic and clinical research with undiminishing enthusiasm until new medicines are found..

Table 1. Receptors identified and their functions

Orphan receptors	Ligands	Functions (diseases)	References
ORL1	Nociceptin/Orphanin FQ	Memory, anxiety	13, 14
HFGAN72	Orexins	Feeding, Narcolepsy	11, 15
GPR10	Prolactin-releasing peptide	Prolactin secretion	12
APJ	Apelin	Blood pressure	16
HG55	Leukotriene D_4	Asthma	9
Edg1	Sphingosine 1-phosphate	Angiogenesis	7
Edg2	Lysophosphatidic acid	Neuronal development	17
OGR1	Sphingosylphosphorylcholine	Ovarian cancer	18
TDAG8	Psychosine	Krabbe's disease	19
G2A	Lysophosphatidylcholine	Immunoregulation	20

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