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Orphans to the rescue: orphan G-protein coupled receptors as new antidepressant targets

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Are orphan G-protein coupled receptors (oGPRs) the next frontier in psychiatric drug targets? Several recent papers suggest that this may finally be the case. G-protein coupled receptors are the target of a large proportion of approved and emerging therapeutic compounds, including for psychiatric illnesses.^{1,2} The oGPRs are receptors for which the endogenous ligand is not known. The first "orphan" receptor human genomic clone, G-21, was discovered in 1987 using low-stringency hybridization with the β 2-adrenergic receptor cDNA.³ G-21 and its rat homologue were subsequently identified as the serotonin-1A (5HT1A) receptor, based on ligand binding studies.^{4,5} The same year, the dopamine-D2 receptor was also identified in this way.⁶ Subsequently, many other GPR-like genes were cloned using polymerase chain reaction-based approaches, but for many their identity was unknown.^{7,8} Since endogenous ligands for these receptors remained unidentified they were classified as oGPRs. "Deorphanization" is the discovery of these endogenous compounds⁸ and has led to the identification of several new families of receptors. These include GPRs for nonclassical ligands, including lipids,⁹ ions, trace amines and adhesion molecules.¹⁰ Recently, oGPRs have been associated with psychiatric illnesses, including major depression, and with responsiveness to antidepressants.^{11,12} These emerging findings highlight their potential importance in susceptibility and treatment of mental illness. Targeting oGPRs may provide novel pharmacological agents for improved treatment of mental illnesses.

Roles of oGPRs in depression

A variety of approaches have implicated oGPRs in depression and antidepressant activity. Typically, gene knockout or viral knockdown of oGPRs (e.g., small interfering RNA) has been used to address their biological function in animal models because of the paucity of specific high-affinity ligands for these receptors. Where available, ligands for oGPRs have also been used in rodent models to test for antidepressant activity. Genomic, proteomic or candidate gene analyses of human tissues have recently revealed changes in oGPR expression levels in blood or postmortem brain from depressed individ-

uals compared with nonpsychiatric controls. Together, these approaches have elucidated several oGPRs as potential biomarkers or drug targets for treatment of major depression. Some recent examples are presented here (Box 1).

GPR56, an adhesion GPR, enhances antidepressant response

Recently, Belzeaux and colleagues¹² screened for blood biomarkers for response to treatment with the serotonin/noradrenaline reuptake inhibitor duloxetine. They found that of 42 positive probes, the adhesion receptor GPR56 showed the greatest increase (19%) compared with nonresponders. This finding was replicated in 2 smaller cohorts receiving either the serotonin reuptake inhibitor citalopram or various antidepressants. In a chronic stress mouse model of depression, responders to fluoxetine showed increased GPR56 expression in blood and prefrontal cortex (PFC). Genetically induced depletion of GPR56 in mouse PFC resulted in a mild depression-like phenotype and resistance to fluoxetine treatment. Conversely, microinjection of GPR56 agonist peptide in the PFC had an acute antidepressant effect, suggesting GPR56 as a potential target to develop novel antidepressants. However, it would be important to first test whether GPR56 activation has a *chronic* antidepressant effect.

GPR158: a pro-depressant glutamate-related GPR

As a second example, Sutton and colleagues¹¹ screened specifically for oGPR expression changes in a chronic stress mouse model of depression compared with unstressed mice. They showed that the PFC expresses a wide variety of oGPRs, with GPR158 being among the most abundant. GPR158 is structurally related to metabotropic glutamate receptors, except that it lacks the extracellular "Venus fly trap" glutamate binding domain.¹³ GPR158 was strongly induced in PFC glutamate neurons and in blood, probably via stress-induced glucocorticoids. It was also increased in the dorsolateral PFC of depressed compared with control human brains. Overexpression of GPR158 in the mouse PFC mimicked the stress-induced depressive phenotype, while GPR158 knockout mice showed reduced

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Box 1: Highlighted oGPRs implicated in major depression*

Orphan	GPR Family	Effect	Species studied	Endogenous ligand	Synthetic
GPR56	Adhesion	Anti Dep; Enh AD	H/R	Collagen III/TG2	Peptide Ag; Non-peptide Antag
GPR158	Adhesion, mGluR-like	Pro Dep	H/R	Proteoglycan GPC4	None
GPR39	Ghrelin-like	Anti Dep; Enh AD	H/R	Zn ²⁺	TC-G 1008 PAM
GPR88	5-HT1B-like	Pro Dep (PD)	R	Unknown	RTI-13951–33 Ag
GPR160	Amine-like	Pro Pain	R	CART	Peptide CART Ag

5-HT1B = serotonin 1B; Ag = agonist; Antag = antagonist; CART = cocaine/amphetamine-regulated transcript; GPR = G-protein coupled receptors; mGluR = metabotropic glutamate receptors; oGPR = orphaned G-protein coupled receptors; PAM = positive allosteric modulator; TG = transglutaminase.

*Shown for the highlighted oGPRs are the receptor family; the effect of the receptor (Pro or Anti) on depression (Dep) alone or in Parkinson disease (PD); on pain; and on enhancing (Enh) antidepressant response (AD); the species studied (human H, rodent R), and endogenous or exogenous ligands.

depressive behaviour and enhanced PFC glutamatergic activity. Thus, inhibition of GPR158 expression or activity may have antidepressant activity, opposite to the role of GPR56.

GPR39: a zinc receptor that mediates antidepressant response

Another oGPR implicated in depression and antidepressant response is the ghrelin-like zinc receptor GPR39. Structurally, GPR39 is in the ghrelin receptor subfamily; however, its peptide ligand remains elusive.⁸ Although GPR39 is activated by zinc, zinc has many targets thus limiting its usefulness in identifying the function of this receptor. Knockout of GPR39 in mice results in anxiety- and depression-like behaviours and resistance to monoamine-enhancing antidepressants, but not to ketamine.^{14,15} GPR39 appears to activate cyclic adenosine monophosphate (cAMP) signalling, and cAMP response element binding protein (CREB)/brain-derived neurotrophic factor (BDNF) levels were reduced in the frontal cortex and hippocampus of GPR39 knockout mice. In human studies, levels of GPR39 protein were reduced in the hippocampus and cortex of suicide compared with control brains.¹⁶ These studies implicate GPR39 in depression and resistance to antidepressant treatment, although its regulation and role needs to be studied in a clinical population.

oGPRs as depression biomarkers

Although GPR56 was identified using a biomarker approach,¹² the small enrichment in the treatment response group limits its utility as a prognostic peripheral biomarker. However, GPR56 expression did not differ between antidepressant responders and nonresponders at admission. It was induced only after 8 weeks of treatment, and more robustly at 30 weeks. Thus, GPR56 cannot be used to predict antidepressant response, but appears to be induced by selective serotonin reuptake inhibitors and serotonin-norepinephrine reuptake inhibitors. For GPR158, the association of its blood levels with depression in humans or mouse models appears to mainly reflect chronic increases in serum glucocorticoids induced by stress.¹¹ Glucocorticoids enter the brain and regulate gene expression via glucocorticoid receptors in both brain and blood with correlated activity. Indeed, many biomarkers simply reflect chronic glucocorticoid load^{17,18} and are thus biomarkers of chronic stress rather than depression per se.¹⁹ In a complementary way, genetic polymorphisms in oGPR genes may also provide blood biomarkers for chronic stress. However, some may be specific

for subtypes of depression. For example, polymorphisms that disrupt the X-chromosome gene *GPR50* have been associated with bipolar depression in a small study in women.²⁰ However, this needs further replication in larger samples, and it is unlikely that single gene polymorphisms will be predictive.

Targeting oGPRs to develop new antidepressants

Because ligands for oGPRs are often lacking, the mechanisms of oGPR actions have been difficult to assess. GPR56 is an adhesion receptor that binds matrix proteins collagen type III and transglutaminase and is implicated in cortical development.¹⁰ Adhesion receptors are activated by self-cleavage of an N-terminal tethered peptide upon binding to synaptic or extracellular matrix proteins. For example, GPR56 binds to collagen III and transglutaminase 2.¹⁰ Thus, these receptor-derived tethered peptides can be used as agonists. However, as peptides they have limited stability, brain permeability, affinity and specificity since they are not constrained at the receptor as is the endogenous peptide. Recently, drug screens have begun to identify nonpeptide ligands for GPR56 including high-affinity antagonists, suggesting that this class of oGPRs may yield pharmacologically useful compounds.²¹

Like GPR56, GPR158 appears to recognize an extracellular matrix component, heparan sulfate proteoglycan GPC4. As an adhesion receptor, GPR158 has been implicated in synaptic contacts between hippocampal mossy fibres and CA3 pyramidal neurons.²² Instead of a glutamate binding site, GPR158 contains calcium-binding epidermal growth factor (EGF)-like and Leu-rich N-terminal domains that may mediate these interactions.¹³ It constitutively recruits RGS7-Gb5 to the membrane to inactivate signalling of inhibitory G-proteins (Gi/Go).^{13,23} GPR158 also prevents cAMP-mediated inactivation of Kv4.2 potassium channels, reducing PFC pyramidal neuronal activity.²⁴ Thus, stress-induced induction of GPR158 mediates inactivation of PFC activity. It may also affect synapses to cortical neurons as seen in the hippocampus, but this has not been reported.

In the case of GPR39, a screen of Chinese hamster ovary cells transfected with human GPR39 led to the discovery of a potent positive allosteric modulator, GPR39-C3/TC-G 1008.²⁵ TC-G 1008 is highly specific to enhance zinc activation of GPR39, but also binds to 5-HT1A receptors.²⁶ Importantly, TC-G 1008 shows persistent antidepressant activity in mice.²⁷ While further ligand optimization for receptor specificity, kinetics and toxicology is needed, GPR39 agonists may provide novel antidepressant treatments.

The future for oGPRs in psychiatry

Identifying the roles of specific oGPRs in mental illness has uncovered additional leads to those discussed here, but many oGPRs remain to be studied. For example, GPR88, which is strongly expressed in the striatum, has been recently implicated in depression-like behaviour in a rat model of Parkinson disease.²⁸ However, high-affinity ligands for GPR88 have yet to be identified.²⁹ The recent brain expression mapping of 78 oGPR genes³⁰ will provide a useful resource to begin to address the roles of oGPRs in neuropsychiatric diseases. Ongoing deorphanization efforts may reveal new drug targets. A case in point, GPR160 has recently been shown to be the CART (cocaine/amphetamine-regulated transcript) receptor, with implications for pain and drug addiction.³¹ Agonists of GPR56 and GPR39 show antidepressant activity in mouse models of depression, with potential as novel antidepressant or augmentation therapies in human depression. On the other hand, antagonists of GPR158 may promote resilience to depression, as in mouse models. However, these oGPRs are widely distributed in the brain and in development, which may lead to unexpected or adverse effects. While their functions can be parsed using knockout approaches in mice, ultimately ligand testing in humans will determine feasibility, safety and efficacy of targeting these receptors. Because the time from deorphanization to drug discovery can exceed 10 years,⁸ these orphans, while promising, will need time to advance in psychiatric treatments. Nevertheless, accumulating evidence on critical roles in psychiatry for this pharmacologically rich family of proteins suggests that these orphans may help rescue the dearth of new lead compounds for treatment of mental illness.³²

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