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Comments

COMMENTS

Gut metabolites: make orphans adopted

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Editor's note

A commentary on "A forward chemical genetic screen reveals gut microbiota metabolites that modulate host physiology".

G protein-coupled receptors (GPCRs) are the largest family of membrane receptors and contain seven-transmembrane helices. GPCRs are versatile membrane receptors and regulate diverse intracellular signaling pathway in response to many extracellular stimuli. Upon activation by ligands, GPCRs couple to intracellular effectors, including G proteins and arrestins,[1] which in turn mediate diverse downstream signaling pathways that shape a broad range of physiological functions. Many well-characterized GPCR ligands/agonists, including light, odors, hormones, neurotransmitters and intestinal metabolites, exert different physiological functions in different cell types and tissues. Hence GPCRs represent readily druggable targets of more than one-third of currently prescribed medications.[2] There are approximately 350 nonolfactory GPCRs, comparable to the number of olfactory GPCRs, but at least one-third of nonolfactory GPCRs are orphan receptors with unknown endogenous or inherent ligands.[3] Thus 'deorphanization' of orphan GPCRs is a major challenge of this field.

The screening of ligands for orphan or unannotated GPCRs is difficult because of the inherent diversity of signal-transducing cascades. To date, functional assays of GPCR activity typically depend on heterotrimeric G protein-coupling,[4] whereas studies indicate that certain GPCRs mediate signal transduction independent of canonical G protein coupling,[5] suggesting this approach is not suitable for GPCRome-wide screening. However, since nearly all characterized GPCRs recruit β-arrestin,[6,7] the measurement of G protein-independent β-arrestin recruitment provides an alternative assay platform. Many GPCR-β-arrestin interaction-based approaches have been documented, including high-content screening (HCS), bioluminescence resonance energy transfer (BRET), enzyme complementation, and transcriptional activation following arrestin translocation (Tango),[8–11] but none of these approaches are routinely performed in a genome-wide, parallel manner. The Tango assays have many advantages, including G protein independence, high signal to background ratio, and stable amplification of relatively small inputs into large output signals.[11] Based on the Tango assay, Kroeze *et al.* developed a method called PRESTO-Tango (parallel receptorome expression and screening via transcriptional output, with transcriptional activation following arrestin translocation), which facilitates rapid, efficacious, parallel, and simultaneous screening of bioactive

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compounds across the entire conventional human GPCRome.[12] By using this unique platform, Kroeze *et al.* validated more than 120 nonorphan human GPCR targets. Furthermore, by screening approved drugs (NIH Clinical Collection of compounds, NCC-1 library) against 91 orphan GPCRs, Kroeze *et al.* confirmed that nateglinide was a selective agonist for only one target, MRGPRX4 orphan receptor, and the HIV protease inhibitor saquinavir selectively activated the BB3 bombesin receptor. Kroeze *et al.* shared PRESTO-Tango as an open-source resource to facilitate the deorphanization of GPCRs.

The human microbiota consists of trillions of bacteria that produce thousands of unique metabolites. Gut microbiota metabolites can influence nearly all aspects of human physiology.[13–15] These metabolites exert local and systematic functions via accumulating in the gut and reaching high concentrations in the serum, respectively.[16] To these metabolites derived from food or bacteria, human GPCRs are pivotal sensors of their small molecules, by selecting chemicals from the thousands of metabolites. Previous studies have identified a variety of microbial metabolite-sensing GPCRs such as GPR43, GPR41, GPR109A, GPR120, GPR40, GPR84, GPR35, and GPR91.[15] GPR41 and GPR43 have been reported to sense short chain fatty acids, such as acetate, propionate and butyrate, to maintain gut homeostasis and regulate macrophage/dendritic cell biology.[13] Tryptophan-derived kynurenic acid acts as a ligand of GPR35 to exert anti-inflammatory function.[13] Long chain omega 3 fatty acids are sensed by GPR40 and GPR120, and inhibit TNFα and IL-6 expression, to suppress inflammation.[13] Most recently, N-acyl amide was reported as an agonist of GPR119 that regulates metabolic hormones and glucose homeostasis.[17] Thus, the thousands of intestinal metabolites produced by the microbiota seem to be a natural pool of GPCR ligands.

Based on recent developments in high-throughput screening of the GPCRome using the platform PRESTO-Tango, Chen *et al.* screened microbiota-derived metabolites for their ability to activate GPCRs, and identified gut commensal metabolites as a rich pool of agonists for both well-characterized and orphan GPCRs. This work was very recently published in *Cell*.[18] In this research Chen *et al.* isolated 144 unique bacterial strains from 11 inflammatory bowel disease (IBD) patients, and cultured these bacteria in a cultivation medium specific for gut commensals as previously described.[19] By using the PRESTO-Tango platform, Chen *et al.* screened the culture supernatants for their capability to activate GPCRs, compared with medium alone. They found that Bacteroidetes and Proteobacteria potently activate the succinate receptor, while Firmicutes, Fusobacteria and Actinobacteria did not, but most other GPCR activation patterns did not correlate with phylogeny. Meanwhile, Chen *et al.* identified more than a dozen bacterial supernatants that activate dopamine receptors (DRDs) or histamine receptors (HRHs), both aminergic receptors, which regulate diverse core physiological process.[20] Among these bacterial isolates, all eight *Morganella morganii* strains activated both DRDs and HRHs, and two *Lactobacillus reuteri* strains activated HRHs. Furthermore, Chen *et al.* found that *M. morganii* produced trace amounts of dopamine and undetectable tyramine, but significant quantities of phenethylamine (PEA), a chemical with mood enhancing properties that can readily cross the blood-brain barrier. Mechanistically, L-Phe, L-DOPA and L-Tyr are the natural precursors for PEA, dopamine and tyramine production, respectively, all catalyzed by the human enzyme aromatic L-amino acid decarboxylase. However, *M. morganii* selectively converted L-Phe to PEA, but not L-DOPA to dopamine or L-Tyr to tyramine. Notably, by using the cAMP response element-secreted human placental alkaline phosphatase (CRE-SEAP) assay, they found that PEA is a full agonist for DRD2-4, but may be a biased agonist for DRD1 and DRD5 that activates G protein-dependent signaling but not β-arrestin recruitment. Chen *et al.* also found another bacteria strain, assigned to the species *B. theta* (*B. theta* C34), that produced the essential amio acid L-Phe, which acts as an agonist for GPR56/AGRG1. Interestingly, *B. theta* C34-produced L-Phe could be efficiently converted to PEA by *M. morganii* C135 *in vitro* and *in vivo*, and thus the metabolic exchange between the two bacterial species contributes to the production of a bioactive trace amine that can affect systemic host physiology.

Consistent with previous reports, Chen *et al.* found that *M. morganii* secreted significant amounts of histamine, and that two *L. reuteri* strains and two Enterobacteriaceae strains also secreted histamine. Supplementation of L-His in the culture medium enhanced the production of histamine by these strains. They further found that *in vivo*, germ-free mice colonized with either *M. morganii* or *L. reuteri*, exhibited high levels of gut histamine production. *M. morganii* primarily colonized the cecum and colon, and caused increased colon motility, which is a well-known physiological function mediated by histamine. In contrast to histamine, the level of PEA accumulation was low in the gut upon *M. morganii* colonization. However, host monoamine oxidase (MAO) enzymes are known to degrade PEA and other biogenic amines in the gut and elsewhere in the body, which may restrict their accumulation both locally and systemically. Indeed, treatment of mice monocolonized with *M. morganii* with an antidepression MAO inhibitor resulted in 'phenethylamine poisoning'. These results suggest that gut bacteria-derived metabolites exert potent physiological effects *in vivo* and demonstrate how gut bacteria can impact responses to medical drugs.

Altogether, Chen *et al.* used host GPCR activation as a lens to parse bioactive metabolites produced by intestinal microbes. They revealed a diet-microbe-host axis where microbiota-derived histamine regulates gut motility, and a tripartite microbe-microbe-host relationship that

results in PEA production. By using a reductionist methodology, Chen *et al*. revealed a metabolite exchange between gut commensals, *M. morganii* and *B. theta*, that is, *B. theta*-derived L-Phe was converted by *M. morganii* to PEA. The reductionist approach is shown to be effective in revealing the metabolic exchanges in a complex microenvironment like the gut that are easily missed out in direct examinations for endpoint metabolites.

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Conflict of interest

The authors declare no conflicts of interest.

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