# Microbiota metabolites as agonists for the orphan receptor GPRC5A

We used chemical proteomics to identify candidate protein targets of indole metabolites in mammalian cells. We discovered that microbiota-derived and synthetic aromatic monoamines can activate recruitment of β-arrestin to the orphan receptor GPRC5A. Specific microbiota species that express amino acid decarboxylases were found to produce aromatic monoamine agonists for GPRC5A.

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## The problem

The human microbiota produces a variety of metabolites that regulate host physiology and disease. Indeed, microbial metabolism of polysaccharides, amino acids, bile acids and synthetic drugs has profound effects on host metabolism, immunity and neurological behavior, and the activity of therapeutics. In particular, dietary aromatic amino acids such as tryptophan, tyrosine and phenylalanine are metabolized by the gut microbiota into a variety of biologically active molecules[1]. Although microbiota metabolites have been shown to engage various classes of cellular proteins, such as pattern-recognition receptors, nuclear receptors and G-protein-coupled receptors (GPCRs), defining the protein targets of microbiota metabolites and determining their precise mechanisms of action is still challenging. Chemical proteomics provides a powerful approach to identifying the metabolite-interacting proteins in cells and characterizing their mechanism(s) of action[2].

## The discovery

We developed photoaffinity reporters of indole-3-acetic acid and tryptamine (Fig. 1), two prominent microbiota metabolites that are detectable in human fecal samples at micromolar levels[3]. We identified many candidate proteins that interact with indole metabolites, including metabolic enzymes, small-molecule transporters, immune sensors and orphan GPCRs. Of note, we found that the indole metabolite photoaffinity reporters can photo-crosslink retinoic-acid-induced protein 3 (RAI3), encoded by *GPRC5A*[4], a class C orphan GPCR associated with inflammation and tumorigenesis. In addition, a PRESTO-Tango assay[5] showed that aromatic monoamines, including tryptamine, phenethylamine and tyramine, can stimulate GPRC5A to recruit β-arrestin. Furthermore, structure–activity relationship studies of aromatic monoamine derivatives identified 7-fluorotryptamine as a more potent synthetic agonist for GPRC5A than tryptamine. Mutagenesis of *GPRC5A* identified N252 and F256 as amino acid residues that act as potential binding sites for aromatic monoamines.

We then evaluated whether GPRC5A agonists can be produced by specific microbiota species and their respective aromatic amino acid decarboxylases, including *Ruminococcus gnavus* (tryptophan decarboxylase), *Morganella morganii* (glutamate or tyrosine decarboxylase) and *Enterococcus faecium* (tyrosine decarboxylase). Phylogenetic analysis of *E. faecium* tyrosine decarboxylase (TyrDC) demonstrated that it is highly conserved in the strains of *Enterococcus* species that are present in human gut microbiota. Metabolomics analysis of wild-type *E. faecium* and a TyrDC-deletion mutant revealed that TyrDC is involved in metabolizing aromatic amino acids to monoamines. Finally, we used CRISPR–Cas9 to knock out *GPRC5A* in the HT-29 colorectal cancer cell line and evaluated changes in gene expression by RNA sequencing. Transcriptional profiling of cells in which the gene encoding GPRC5A was knocked out suggested that this unique GPCR has key roles in immune and cancer signaling.

## The implications

Delineating the mechanisms of action of specific microbiota species and metabolites is crucial for understanding their impact on host physiology and disease. Metabolite-sensing GPCRs mediate the chemical dialog of host–microorganism interactions. Aberrant expression of *GPRC5A* has been associated with multiple human cancers. Our discovery that indole metabolites bind to GPRC5A and that aromatic monoamines activate GPRC5A-mediated recruitment of β-arrestin provides an important first step toward the development of more-effective pharmacological tools and characterization of the interactions between specific microbiota metabolites and GPRC5A.

In contrast to the large extracellular agonist-binding region of canonical class C GPCRs, GPRC5A has a short N-terminal sequence that is unlikely to bind ligands involved in classic G-protein-mediated signaling. It is possible that aromatic monoamines are not potent enough agonists to induce additional GPRC5A signaling beyond recruitment of β-arrestin. Alternatively, engagement of other GPCRs by aromatic monoamines may interfere with GPRC5A-specific signaling. It is also possible that aromatic monoamines act as allosteric agonists and require additional ligands or protein components to fully activate GPRC5A signaling.

The future discovery and development of agonists that are more potent and selective may help to elucidate how GPRC5A signaling is regulated. In addition, applying synthetic agonists or aromatic-monoamine-producing bacteria in different mouse models may help to clarify the roles of GPRC5A signaling in inflammatory diseases and colorectal cancer.

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## EXPERT OPINION

"In this elegant study, the authors de-orphan the G-protein coupled receptor GPRC5A using a photoaffinity labeling probe that resembles its microbiota-derived ligands, identify gut microbiome bacteria that produce these ligands, develop synthetic agonists that are more potent than these ligands, and address the function of this GPCR. In sum, this study is novel and impactful because it de-orphans a GPCR and finds that microbial ligands can activate this receptor, which may be involved in cancer and inflammation." **Pamela V. Chang, Cornell University, Ithaca, NY, USA.**

## FIGURE

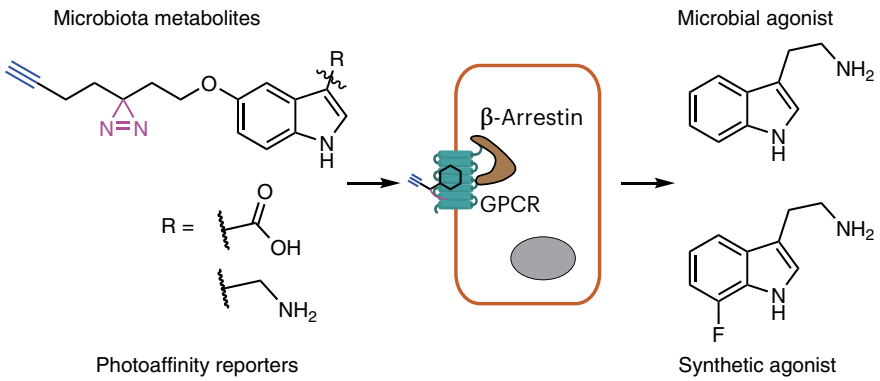


**Fig. 1 | Chemoproteomics of microbiota-derived indole metabolites reveals aromatic monoamine agonists of GPRC5A.** Photoaffinity reporters (left) of indole-3-acetic acid and tryptamine photo-crosslink GPCRs in mammalian cells. Specific microbiota species and their respective aromatic amino acid decarboxylases produce aromatic monoamines that stimulate recruitment of β-arrestin to GPRC5A. Synthetic aromatic monoamine derivatives such as 7-fluorotryptamine (bottom right) function as more-potent GPRC5A agonists than microbial aromatic monoamines such as tryptamine (top right). © 2023, Zhao, X. et al.

## BEHIND THE PAPER

This study of microbiota-derived indole metabolites was initiated as part of our laboratory's efforts to determine the functional roles of microbiota metabolites, including peptidoglycan, short-chain fatty acids and bile acids. We have identified many interesting candidate host proteins with which these metabolites interact but chose GPRC5A for functional studies due to its 'orphan' status and aberrant expression in various cancers. The PRESTO-Tango assay, which is available as an open-source resource, was one of the key methods we used to identify the aromatic monoamine agonists for GPRC5A. We are very grateful for the generous gift of research materials from the scientific community and our collaborators. Although the start of the COVID-19 pandemic in early 2020 forced us to slow down our research into other synthetic aromatic monoamine derivatives and cultures of microbiota species, it gave us the time to discuss and prepare this study. **H.C.H. & X.Z.**

## REFERENCES


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   **This paper presents PRESTO-Tango, a cell-based assay for detecting the recruitment of β-arrestin by GPCRs.**


## FROM THE EDITOR

"This study provides a nice interplay between microbial metabolism and GPCR signaling and highlights the strength of chemoproteomics in the identification of mechanisms of action for microbial metabolites." **Editorial Team, *Nature Chemical Biology*.**