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G PROTEIN-COUPLED RECEPTORS

Expanding the detection of GPCR activation

High-throughput cell-based assays for detecting G protein-coupled receptor (GPCR) signalling are widely used in drug discovery, reflecting the importance of this large family of receptors as therapeutic targets. However, although such assays have been established to detect G protein-mediated receptor signalling with a Gα protein subunit from three out of the four major subfamilies — $G\alpha_s$, $G\alpha_i$ and $G\alpha_q$ — they cannot be applied to all $G\alpha_{12/13}$-coupled receptors, which has limited the characterization of such receptors. Now, Inoue and colleagues have developed a high-throughput assay that can accurately detect the activation of $G\alpha_{12/13}$-coupled receptors as well as those signalling through other Gα proteins.

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In a previous study, the authors had observed that the ectodomain of transforming growth factor-α (TGFα) undergoes cleavage (shedding) following the activation of the GPCR lysophosphatidic acid receptor 6 (LPA_6), through a pathway that is dependent on $G\alpha_{12/13}$, but this LPA_6 activation was not detected by existing assays. This led them to consider whether measuring TGFα shedding in GPCR-expressing cells could be useful in detecting $G\alpha_{12/13}$-mediated GPCR signalling as well as other forms of Gα-mediated signalling. The basis of their TGFα shedding assay is as follows: following ligand binding, GPCR activation induces the shedding of the ectodomain of membrane-bound TGFα; tagging TGFα with alkaline phosphatase and quantifying its release can thus enable the detection of GPCR activation.

Using this assay, the authors first screened 116 GPCRs with established ligands and detected the activation of 75 GPCRs, including 14 GPCRs that were previously unknown to couple with $G\alpha_{12/13}$. Furthermore, they noted that $G\alpha_q$- and $G\alpha_{12/13}$-coupled GPCRs induced stronger TGFα shedding responses, whereas $G\alpha_s$- and $G\alpha_i$-coupled GPCRs induced weaker or no responses. TGFα shedding was also enhanced in cells that expressed chimeric Gα proteins or the promiscuous $G\alpha_{16}$ protein (which can couple with multiple GPCRs). The authors therefore generated chimeric Gα proteins expressing $G\alpha_q$ and $G\alpha_{12/13}$ backbones to broaden the scope of GPCR detection.

Plasmid-based co-expression of five such chimeric Gα proteins and the $G\alpha_{16}$ protein ultimately enabled the detection of GPCR activation for 104 out of the 116 GPCRs tested — the highest fraction ever achieved with a single assay. Moreover, using the TGFα shedding assay to screen ligands for orphan GPCRs, the authors identified lysophosphatidylserine as a ligand for three orphan GPCRs and confirmed that these receptors are $G\alpha_{12/13}$-coupled GPCRs.

This method can also be used to ascertain whether GPCR–ligand binding induces agonism, inverse agonism or antagonism (which has been difficult to determine using conventional assays). The TGFα shedding assay is therefore a versatile tool for studying GPCR activation; most importantly, by accurately detecting a broad range of GPCRs, this method overcomes several limitations of existing assays and could thus be used to study many GPCRs that have not yet been effectively characterized.

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ORIGINAL RESEARCH PAPER Inoue, A. *et al.* TGFα shedding assay: an accurate and versatile method for detecting GPCR activation. *Nature Methods* **9**, 1021–1029 (2012)