RESEARCH HIGHLIGHT

ONE-GO: Direct detection of context-dependent GPCR activity

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G protein-coupled receptors (GPCRs) represent some of the most important drug targets in the human genome, however, our understanding of these receptors has primarily been confined to systems that require the overexpression of target receptors. In a recent study published in *Cell*, Janicot et al. introduced a universal biosensor platform capable of detecting GPCR signaling at endogenous levels, which facilitates its application across various cell lines, including primary cells, thereby improving our understanding of context-dependent GPCR activity and system bias.

G protein-coupled receptors (GPCRs) constitute the largest superfamily of transmembrane proteins in the human genome. They regulate diverse intracellular signaling cascades in response to numerous extracellular stimuli such as hormones and neurotransmitters. While GPCRs are targeted by over 33% of currently marketed therapeutic drugs and high-resolution structures of most non-olfactory receptors have been elucidated, a comprehensive understanding of the mechanisms underlying GPCR signaling triggered by drugs remains elusive.[1,2] This is due to potential crosstalk in native cells and the variability in signaling outcomes depending on the cellular system. This lack of understanding may be attributed to the scarcity of tools available for researchers to study GPCRs in their native environment. Many existing tools rely on the overexpression of target receptors, potentially distorting findings and masking any crosstalk or modulation that would occur under physiological conditions. Moreover, the reliance on overexpression limits researchers to engineered and immortalized cell lines capable of sustaining such high protein levels, thereby limiting our understanding of GPCR signaling to these artificial systems. Different cellular systems, however, are hardwired to respond differently to the same cellular stimuli, a phenomenon known as system bias or context-dependent signaling.[3] The ability to study these systems using endogenous levels of proteins along with different cell lines is therefore pivotal to understanding these mechanisms and thus producing effective and translatable drugs.

In the classical GPCR signal transduction scheme, stimulus-driven receptor activation leads to the coupling of active receptors to their cognate GDP-bound G proteins resulting in the exchange of GDP to GTP and subsequent dissociation of Gα-GTP and free Gβγ, detection of which has recently been employed for the direct measurement of GPCR activity. The activated Gα subunit then triggers specific signal transduction pathways depending on its type ($G\alpha_s$, $G\alpha_{i/o}$, $G\alpha_{q/11}$, or $G\alpha_{12/13}$), stimulating or inhibiting the production of secondary messengers such as cAMP that have also been utilized to measure GPCR activity indirectly.[4] Individual Gα subunits act as GTPases hydrolyzing GTP to GDP, after which Gα-GDP associates back with Gβγ forming GDP-bound G proteins, and the cycle repeats. Additionally, GPCRs can signal through β-arrestins and other transducers. Indirect measurement of GPCR activity has long been a popular approach, leading to the development of several successful drugs currently on the market. However, the amplified and indirect nature of such assays, as well as their reliance on specific cell types, have contributed to a low rate of turnover of drug leads to the market.[5]

Over the last decade, several assays have been employed to detect direct GPCR activity by utilizing G protein dissociation, based on bioluminescence or fluorescence resonance energy transfer (BRET or FRET).[6] TRUPATH, for example, is a well-optimized biosensor platform that directly detects the dissociation of Gα-Rluc8 (donor) and Gβ/Gγ-GFP2 (acceptor), which covers 14 different Gα variants out of 16 non-visual G protein transducers in human cells.[7] TRUPATH, however, is sensitive to conformational transitions in Gα and requires overexpression of exogenous G protein subunits, which may significantly perturb the system under investigation. Other biosensors, namely a BRET biosensor with ER/K linker and YFP (BERKY)[8] and an Effector Membrane Translocation Assay (EMTA),[9] have been designed to detect Gα-GTP rather than measure Gα/βγ dissociation. A notable restriction for both biosensors is the lack of direct detection for $G\alpha_s$ proteins. Additionally, EMTA biosensors may interfere with signaling, while BERKY suffers from a low dynamic range.

In their recent study, Janicot et al.[10] addressed some of these limitations by introducing a ONE vector G protein Optical (ONE-GO) biosensor (Fig. 1). This biosensor is a single plasmid system that includes a YFP-fused Gα (acceptor) and membrane-anchored nanoluciferase (Nluc) (donor) fused with a peptide or protein domain exhibiting high specificity for Gα-GTP but not towards Gα-GDP. This design gives ONE-GO several critical advantages over many other assays that currently exist. It measures direct GPCR activation without relying on overexpression, making it applicable to virtually any cellular system. The assay has been successfully applied across six different primary cell types from two species, utilizing all four G protein families and ten different receptors supporting the context-dependent activity of GPCRs such as the selectivity of G protein families depending on cell types or disease-like states. Of the many advantages, a notable limitation of this assay is its incomplete G protein catalog, covering only ten isoforms. These biosensors, however, present an open-source platform facilitating their wider use and future development. Furthermore, although the authors demonstrated that only a low amount of exogenously expressed Gα is needed and it does not interfere with downstream signaling, the presence of chimeric Gα in the system

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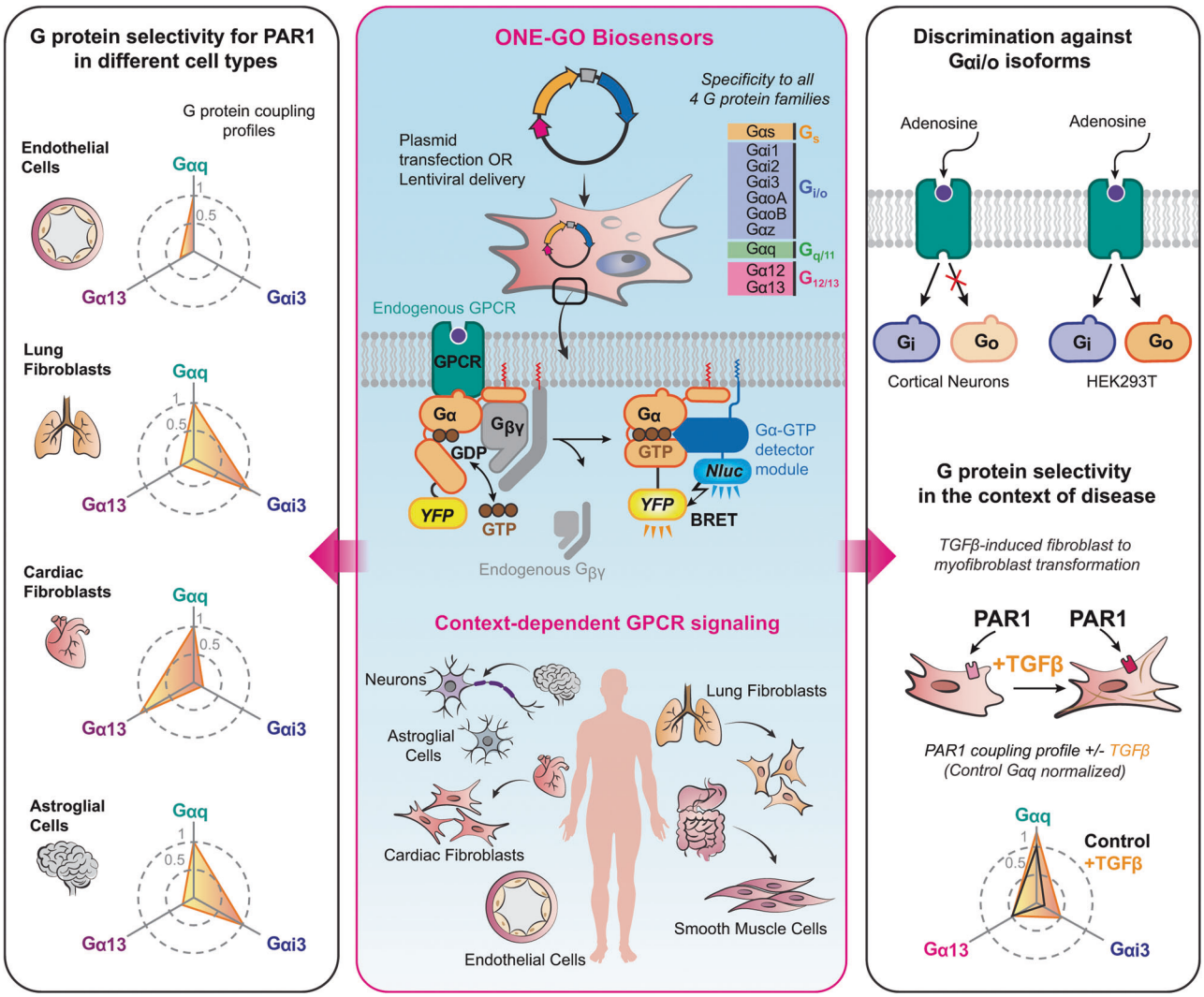

Fig. 1 Design and applications of the ONE-GO biosensor. Middle, the ONE-GO biosensor is encoded on a single plasmid that can be transfected or delivered via a lentivirus into a variety of cell lines including primary cells, enabling direct detection of context-specific G protein signaling at endogenous receptor levels. Left, ONE-GO detects bias in G protein selectivity for PAR1 in different cell types. Right, ONE-GO reveals discrimination across $G_{i/o}$ isoforms by neuroinhibitory receptors in primary neurons and identifies the transformation of G protein selectivity of PAR1 in the context of disease.

could generate biased outcomes compared to native Gα. Nevertheless, this biosensor outperformed other assays by producing stronger signals, enabling the detection of endogenous GPCR responses without compromising the robust dynamic range.

In conclusion, Janicot et al.[10] have developed a universal biosensor for the direct measurement of context-dependent GPCR activity and demonstrated its versatility in revealing intricacies of GPCR signaling in primary cells. The open-source nature of the ONE-GO biosensor should facilitate its further optimization and extension to cover remaining Gα isoforms and other transducers. Taken together, this assay holds potential to advance our understanding of diverse cell signaling mechanisms and contribute to the discovery of drugs with desired profiles of signaling activity.

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ADDITIONAL INFORMATION

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