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REVIEW

Genome-wide pan-GPCR cell libraries accelerate drug discovery

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Abstract G protein-coupled receptors (GPCRs) are pivotal in mediating diverse physiological and pathological processes, rendering them promising targets for drug discovery. GPCRs account for about 40% of FDA-approved drugs, representing the most successful drug targets. However, only approximately 15% of the 800 human GPCRs are targeted by market drugs, leaving numerous opportunities for drug discovery among the remaining receptors. Cell expression systems play crucial roles in the GPCR drug discovery field, including novel target identification, structural and functional characterization, potential ligand screening, signal pathway elucidation, and drug safety evaluation. Here, we discuss the principles, applications, and limitations of widely used cell expression systems in GPCR-targeted drug discovery, GPCR function investigation, signal pathway characterization, and pharmacological property studies. We also propose three strategies for constructing genome-wide pan-GPCR cell libraries, which will provide a powerful platform for GPCR ligand screening, and facilitate the study of GPCR mechanisms and drug safety evaluation, ultimately accelerating the process of GPCR-targeted drug discovery.

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1. Introduction

GPCRs, a superfamily that comprises over 800 members in the human genome, including approximately 400 olfactory receptors (ORs), emerge as an exceptionally promising category of therapeutic targets[1]. They play crucial roles in drug screening and elucidating pathogenesis[2], are considered "star" targets in drug development. Currently, about 40% of clinical drugs are targeting GPCRs[3]. According to the latest data from the GPCR database (https://gpcrdb.org/drugs/drugbrowser), there are 475 FDA-approved GPCR drugs, and 483 GPCR drugs are in clinical trials[4]. An overview of recently approved drugs targeting GPCRs in the last five years and their applications in treating various diseases are listed in Table 1. To date, only about 103 GPCRs have been recognized as drug targets[5], over 100 non-olfactory GPCRs[6] and approximately 90% of ORs are still orphan receptors (oGPCRs)[7]. There are numerous potentials for GPCRs as drug targets and they largely remain unexplored.

GPCRs feature an extracellular N terminus, an intracellular C terminus, and seven transmembrane helixes connected by extracellular and intracellular loops[8,9]. These structural elements play essential roles in GPCR function and signal transduction. GPCRs initiate signal transduction by engaging with the heterotrimeric G protein composed of Gα, Gβ, and Gγ subunits[10]. Upon GPCR activation, the Gα subunit binds to guanosine triphosphate (GTP) and dissociates from the G$\beta\gamma$ dimer, initiating downstream signaling cascades[11]. GPCRs and the related signaling transducers are integral to human physiology and prime targets for pharmacological intervention, positioning them at the cutting edge of drug discovery[12,13]. Meanwhile, the intricate signaling networks, and the complex interactions between receptors and diverse ligands, present significant challenges in the GPCR research field[14].

Table 1 FDA-approved drugs targeting GPCRs in the last five years.

Family	Target	Drug	Clinical indication	FDA approval year
Class A	ADRB3	Vibegron	Overactive bladder disease	2020
	GnRHR	Relugolix	Prostate cancer	2020
	MC4R	Setmelanotide acetate	Obesity	2020
	S1PR1	Ozanimod hydrochloride	Multiple sclerosis	2020
	S1PR5			
	DRD2	Amisulpride	Schizophrenia	2020
	DRD3			
	C5AR	Avacopan	Wegener's granuloma	2021
	Adrenoceptors	Ephedrine hydrochloride	Anaesthesia	2021
	GnRHR	Relugolix	Smooth muscle tumor	2021
	S1PR1	Ponesimod	Multiple sclerosis	2021
	PTGER2	Omidenepag isopropyl	High intraocular pressure Open-angle glaucoma	2022
	AVPR	Terlipressin acetate	Hepatorenal syndrome	2022
	OX1R	Daridorexant hydrochloride	Sleeping and sleep disorders	2022
	OX2R			
	S1PR1	Etrasimod arginine	Ulcerative colonitis	2023
	S1PR4			
	S1PR5			
	5-HT1A	Gepirone hydrochloride	Major depressive disorder	2023
	CXCR4	Motixafortide	Haematopoietic stem cell transplantation	2023
	NK3R	Fezolinetant	Vasodilatation	2023
	AT1	Sparsentan	Immunoglobulin A nephropathy	2023
	ETAR			
	CXCR4	Mavorixafor	WHIM syndrome	2024
	ETAR	Aprocitentan	Hypertension	2024
	ETBR			
Class B1	CGRPR	Rimegepant sulfate	Migraineur	2020
	CGRPR	Atogepant	Migraineur	2021
	GCGR	Dasiglucagon	Hypoglycaemia	2021
	GIPR	Tirzepatide	Type 2 diabetes mellitus	2022
	GLP-1R			
	CGRPR	Zavegepant	Migraineur	2023
Class C	GPRC5D	Talquetamab	Multiple myeloma	2023

The listed drugs (approved from 2020 to 2024) are from the Drugs@FDA database (https://www.accessdata.fda.gov/scripts/cder/daf/index.cfm), and cross-referenced in the Drugbank (https://go.drugbank.com/), pharmacodia (https://www.pharmacodia.com/homeH5.html), and pharnexcloud database (https://www.pharnexcloud.com/). The abbreviation of the targets refers to the GPCR database (https://gpcrdb.org/drugs/drugbrowser).

Cellular expression systems play crucial roles in pharmaceutical research, enabling the investigation of complex mechanisms underlying GPCR-drug interactions, including binding modes and signaling transduction mechanisms. A range of recent reviews have shown that developing GPCR overexpressed cell lines enables a comprehensive examination of their structure[15], function[16], pharmacological characteristics[17], and downstream signaling pathways[18]. For instance, Thorens et al. conducted stable transfection of the glucagon-like peptide-1 receptor (GLP-1R) using fibroblast cells and promoted the identification of the agonist and antagonist targeting GLP-1R[19]. Similarly, Pronin et al. demonstrated that the ectopic expression of olfactory receptor family 51 subfamily E member (OR51E1 and OR51E2) inhibits proliferation and promotes cell death in lymph node carcinoma of the prostate (LNCaP) cells[20]. These studies highlight the importance of specialized cell lines in drug screening and in the elucidation of GPCR functions and their implications in disease.

Employing cutting-edge biotechnological techniques, specialized cell lines can be engineered for GPCR research, facilitating the identification of potential drug targets[21]. Genome-wide libraries, encompassing a diverse array of genetic components and sequences, serve as essential tools and resources for functional genomics research[22]. These transgenic cell libraries can advance the understanding of gene functions, identification of drug targets, exploration of disease models, and elucidation of disease mechanisms. This review presents a systematic comparison and analysis of various methodologies for cell line development and functional studies. Based on these insights, three strategic approaches were proposed to establish genome-wide pan-GPCR cell line libraries. This initiative aims to create a robust foundation for GPCR-targeted drug screening and enhance our understanding of GPCR-related disease mechanisms.

2. Important roles of cellular expression systems in GPCR research

2.1. *Applications and limitations of current cell expression systems in GPCR research*

Significant advancements in genetics, proteomics, and cell engineering have propelled the progress of life sciences and drug development[23]. A common strategy employed in genetic function studies involves cloning genes into expression vectors, introducing them into host cells, and performing functional analyses[24]. GPCRs are renowned for their pivotal physiological roles and status as versatile drug targets[25]. However, the inherent hydrophobicity, structural instability, and conformational dynamics of GPCRs make it challenging to determine their structures, characterize their functions, and discover GPCR-targeted drugs[26,27]. Therefore, the selection of appropriate cell lines and expression systems is crucial for the investigation of GPCRs.

Structural determination plays a pivotal role in deciphering the molecular architecture of GPCRs and lays a foundation for drug design[28,29]. Obtaining a sufficient amount of properly folded and functionally intact purified proteins is important to accurately delineate the three-dimensional structure of GPCRs[30]. Insect cell systems are widely applied for GPCR expression in structural investigations[31], because they can efficiently express recombinant proteins and form stable proteins suitable for structural analysis techniques such as crystallography and cryo-electron microscopy[32]. For instance, the molecular structures of 5-hydroxytryptamine (5-HT) receptors (5-HT_{1B}, 5-HT_{2B}) bound to antimigraine, antipsychotic, antidepressant, and appetite suppressant drugs were elucidated by using *Spodoptera frugiperda* (*Sf9*) cells[33,34]. This discovery provides a valuable foundation for the design of safer and more effective drugs.

Understanding the function and characteristics of GPCRs is the foundation for exploring their targeted drugs. The cell heterologous expresses the GPCRs that researchers are interested in as the major tools for GPCR functional characterization[35]. Mammalian cell lines are valued for their ability to carry out a broad spectrum of post-translational modifications (PTMs), these are essential for the accurate folding, localization, and functionality of GPCRs[36]. Yeast systems, characterized by parallels to mammalian signaling pathways, rapid proliferation, and economic advantages, are optimal for exploring GPCR engagements with intracellular transducers[37]. However, they have distinct glycosylation patterns from mammalian proteins, making them less suitable for maintaining the activity of glycosylation-dependent proteins[38]. Insect cell expression systems are commonly applied for studying early GPCR ligand recognition and signaling[39,40]. Lacking some mammalian-specific PTMs such as glycosylation and complex protein folding makes the generated heterologously expressed GPCRs good for investigation of the functions of these specific PTMs[41,42].

Drug screening in mammalian cells like human embryonic kidney (HEK) 293T, Henrietta Lacks (HeLa), and Chinese hamster ovary (CHO) are commonly used for their native GPCR processing[43], signaling, metabolism, and genetic tractability, making them ideal for GPCR studies and drug discovery[44]. Stable and transient transfections are effective methods for delivering GPCRs into mammalian cells[45]. Easy to operate and fast to get large amounts of proteins, the transient transfection has been widely applied for heterologous expressing proteins in many GPCR research, like structure determination and functional assays[46,47]. However, it is not ideal enough for long-term GPCR studies due to the unstable gene expression and the possibility of gene loss[48]. Stable transfection enables sustained gene expression and is preferred for in-depth GPCR research, such as disease mechanism investigation and drug discovery[49,50]. Thus, optimized stable transfection techniques often result in long-term stable production of the target protein and prolonged exogenous gene expression compared to transient methods[51,52].

2.2. *Important roles of genome-wide cell libraries in GPCR research*

Large-scale transgenic cell libraries are pivotal in exploring gene functions, target−ligand interactions, and signal regulatory mechanisms. These libraries enable us to conduct functional screening and identify genes with specific biological functions[53], explore the interactions between genes and metabolites by integrating with HTS techniques[54], and study the functions of specific genes employing gene-editing technologies such as CRISPR/Cas9 for knock-out or knock-in experiments. Notable contributions, including the Arabidopsis T-DNA insertion mutant library, have significantly advanced gene function and functional genomics studies[55,56]. The mouse gene mutant library (PBmice), has provided numerous resources for the identification of genes associated with various diseases[57]. The cell line database (CLDB, http://bioinformatics.istge.it/hypercldb) contains over 6000 human and animal cell lines, which improve the reproducibility and collaboration of cell line research, and lay the foundation for clinical translation[58]. These studies indicate that the large-scale library would facilitate the identification of potential drugs[59].

Cellular-level screening plays an important role in the initial stages of drug discovery and sheds light on the diverse roles of GPCRs in various biological processes. Similarly, large-scale GPCR research has unveiled new ligand interactions and their complex roles in cellular signaling. For instance, Jones et al. presented an HTS platform consisting of a range of GPCR overexpressed HEK293T cells for screening chemical libraries against multiple GPCRs, that are heterologously expressed in HEK293T cells[60]. They successfully mapped 39 ORs to 181 odorants and identified 79 previously unreported interactions. Bauknecht et al. successfully identified 19 ligands by screening 126 neuropeptides against 87 GPCRs in *Platynereis dumerilii*[61]. Additionally, Ahmed et al.[62] constructed a GPCR overexpression cell library containing 379 human ORs by employing the GloSensor real-time adenosine monophosphate (cAMP) detection assay, and they identified the OR5AN1 and OR1A1 as receptors for the musk odorant muscone. These significant advancements demonstrate that large-scale cell-based screening plays an important role in GPCR-targeted drug discovery, as well as the elucidation of disease mechanisms.

The genome-wide cell-based study plays a pivotal role in drug screening, providing valuable insights into the mechanisms of genetic interactions and PTMs. Recent studies have developed a genome-wide knockout library, that aids in identifying essential genes for cancer growth and potential targets for human immunodeficiency virus (HIV) treatment[63,64]. Similarly, Tromp et al. conducted a genome-wide CRISPR/Cas9 library screen in U937 human monocytic cells, and used it successfully revealed PTM pathways involved in the sulfation and sialylation of leukocidin receptors[65]. Additionally, the genome-wide cell library can facilitate the target identification and potential side-effects elucidation. An example is fingolimod (FTY720), a then-novel immunosuppressant that has the side effect of bradycardia. Sanna et al.[66] screened the immunosuppressant target of FTY720, sphingosine 1-phosphate receptor 1 (S1PR1), and developed a new generation of immunosuppressants without side-effect, including siponimod, ozanimod, and etrasimod.

These advancements demonstrate the pivotal role of comprehensive libraries in drug discovery, target identification, and the elucidation of complex biological mechanisms, including those underlying the diverse functions of GPCRs. Our lab recently conducted large-scale screenings of Shuxuetong, a Chinese herb-based intravenous formulation, in CHO-dependent transgenic GPCR cell libraries to explore the potential targets for stroke treatment, and uncovered that the GPCRs chemokine (C−C motif) receptor 1 (CCR1), relaxin family peptide receptor 3 (RXFP3) and dopamine receptor D3 (DRD3) are the potential targets to bioactive compounds in Shuxuetong (Jiayu Liao, unpublished). Consequently, the establishment of genome-wide pan-GPCR cell libraries, combined with HTS methods and functional evaluation systems, is critical for the rapid screening and validation of potential drug molecules.

3. Application of GPCR cell lines in high-throughput screening

3.1. GPCR cell lines facilitate ligand binding-dependent high-throughput screening

GPCRs can recognize and bind to various ligands by interacting with specific pockets in the transmembrane helices[5]. According to the activity and binding sites, GPCR ligands are mainly classified as follows: agonists that bind to the orthosteric site and activate the receptor, competitive antagonists that compete with agonists for the orthosteric site and GPCR activity, non-competitive antagonists that inhibit receptor activity by binding to allosteric sites, partial agonists that bind to the orthosteric site and induce a smaller maximum biological response compared to full agonists, inverse agonists that bind to the same orthosteric site as agonists but produce the opposite effect, allosteric agonists that activate the receptor by binding to allosteric sites. This type of classification reflects the complexity and diversity of GPCR functions, offering a wide range of strategies and targets for drug discovery and disease treatment[67]. GPCR ligand binding experiment is a key step to screen and understand the mechanism of GPCR in drug research and development.

Several ligand binding-dependent HTS assays are available for GPCR ligand screening. The commonly used assays for analyzing the biomolecular interactions include fluorescence polarization (FP)[68], surface plasmon resonance (SPR)[69], nanoluc-bioluminescence resonance energy transfer (NanoBRET)[70], scintillation proximity assay (SPA)[71], and tag-lite assays[72]. SPA can be employed for GPCR screening, but the high cost and safety concerns in using radiolabeled chemicals largely limited its wide application[73,74]. In addition, cell membrane chromatography (CMC)[75,76], and affinity mass spectrometry (MS) are widely utilized for HTS of GPCR-ligand binding in proteomics[77].

GPCR heterologous expression systems play important roles in performing these GPCR binding assays, like providing purified heterologous GPCRs or cells that highly express GPCRs on the membrane[78]. For example, HEK293T is a widely used GPCR HTS cell line, which expresses 75 endogenous GPCR[79]. To ensure the reliability of the results in these HTS assays, the cell lines that stably express GPCR are often needed.

At present, FP and MS technology are the most widely used binding-dependent HTS methods for GPCRs. For instance, Heine et al. verified that 12 candidate ligands of neurotensin receptor type 1 (NTSR1) from a compound library of 1272 compounds using NTSR1 cells stably expressed in HEK293T cells[80]. Qin et al. utilized the Bac-to-Bac baculovirus expression system to stably express the 5-hydroxytryptamine 2C receptor (5-HT_{2C}R) and GLP-1R in *Sf9* cells, by screening a small compound library by using affinity MS, they successfully identified some 5-HTR antagonists and four GLP-1R positive allosteric modulators[81]. Moreover, this lab successively constructed transgenic *Sf9* cells expressing the GPR52 and adenosine A2A receptor (A_{2A}AR), and by employing affinity-MS screening ligands they ultimately identified some negative allosteric modulators of GPCR[82,83]. NanoBRET and Tag-lite assays are more sensitive fluorescence techniques, widely used to detect GPCR-ligand interactions[84]. For instance, Killoran et al. constructed 19 transgenic HEK293T-derived cells expressing HiBiT-GPCR, and evaluated the concentration-dependent binding with these 19 GPCRs and related ligands by NanoBRET technique[85]. The tag-lite analysis combines homogeneous time-resolved fluorescence (HTRF®) detection with SNAP-tag® and integrates covalent labeling technology[85]. Belhocine et al.[86] constructed transgenic HEK293T cells expressing SNAP-tag-GPCR and studied the effects of seven different agonists and seven positive allosteric modulators on 34 GPCRs using the Tag-lite method. In addition, CMC is an effective method to study the affinity characteristics of drug membrane receptors. Wang et al. constructed HEK293T-derived cell lines stably

expressing alpha1A or alpha1B-adrenergic receptors (ARs) and used them to evaluate the interaction of the CMC model between ligand and subtype receptor of GPCR[87].

3.2. *Application of cell lines to measure GPCR activity*

Today, the major experimental GPCR HTS approaches primarily rely on assaying GPCR activities through monitoring GPCR signaling pathways. Detection of the GPCR signaling pathway mainly involves changes in the concentration of intracellular second messengers following ligand binding in cell lines[78]. This concentration change is caused by the binding of GPCR to ligand activating Gα, which in turn triggers intracellular signaling pathways. Among them, Gα_s and Gα_i regulate the activity of adenylate cyclase (AC), which converts adenosine triphosphate (ATP) to cAMP and thus regulates its level. Gα_q activates phosphatidylinositol phospholipase C, which catalyzes the catabolism of phosphatidylinositol 4,5-bisphosphate (PIP2) into diacylglycerol (DAG) and 1,4,5-trisphosphatidylinositol (IP3) and ultimately induces endoplasmic reticulum Ca^{2+} release (Fig. 1A). In addition, the recruitment and subsequent desensitization of β-arrestins highlights distinct GPCR signaling pathways and is therefore also frequently reported to detect GPCR ligand binding (Fig. 1B). Screening based on signaling pathways can provide information that may not be available in receptor-binding assays, such as distinguishing between full agonists, partial agonists, and inverse agonists. This usually requires heterologous expression of GPCR in engineered cells, followed by fluorescent assays, reporter genes including Tango assay, and biosensors to report ligand binding[88].

Applying fluorescent or absorbent dyes to label secondary messages Ca^{2+} or cAMP is a common approach to monitor GPCR activation. The commonly used dyes include Calcium-AM, Fluo-4, Fura-2, and cAMP-Glo™ Assay (cAMP-Glo). To improve the sensitivity of drug action detection, the specificity of screening, and to exclude the interference of endogenous GPCR activation, specific GPCRs are usually chosen to be heterologously expressed in cells. Smith et al. heterologous expressed the M_4 muscarinic acetylcholine receptor in CHO cells and then screened 360,000 small molecule libraries for M_4 ligands using a calcium dye assay. This assay identified 25 agonists, 4 orthosteric modulators, and 41 antagonists of the M_4[89].

Introducing reporter protein-encoding sequences into the signaling pathway has been widely employed to assess the degree of activation or repression of the promoter through luminescence, fluorescence, or optical readout monitoring. Some commonly used reporter genes include chloramphenicol acetyltransferase (CAT), β-galactosidase (GAL), β-lactamase (LAC), luciferase (Luc), and green fluorescent protein (GFP). Yaginuma et al. co-transfected cells with GLP-1R and a reporter gene[90]. By monitoring the fluorescence resulting from GLP-1R activation, a new ligand for human GLP-1R was identified among 400 peptides through visualization[90].

In addition, researchers have also developed the Tango assay based on the β-arrestin signaling pathway. One of the major advantages of Tango is that it stably expresses a tetracycline-

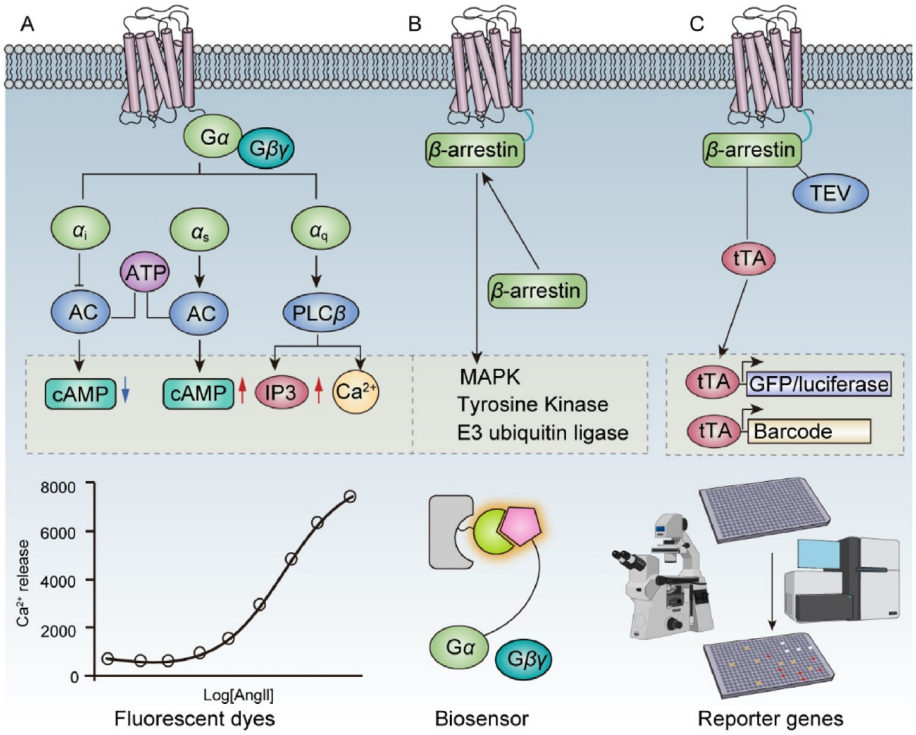

Figure 1 High-throughput GPCR assays based on different signaling pathways. (A) Activation of Gα_s up-regulates AC, leading to an increase in cAMP levels. Activation of Gα_i down-regulates AC, leading to a decrease in cAMP levels. Activation of Gα_q up-regulates Ca^{2+} levels, which can be measured by fluorescent dye, reporter genes, and biosensors. (B) Recruitment of β-arrestins leads to additional signaling pathways that can be measured by fluorescent dye, reporter genes, and biosensors. (C) In the Tango assay System, activation of the GPCR leads to recruitment of the β-arrestins, cleavage of the GPCR-linked tTA by a protease linked to the β-arrestins, and release of the transcription factor that ultimately leads to expression of the reporter gene.

controlled luciferase reporter gene-dependent transcriptional activator (tTA), and allows for the assessment of the GPCR activity status by fluorescence intensity measurements (Fig. 1C). In 2015, this experiment was first extended to >300 GPCRs by adding various tagged proteins and co-transfecting GPCRs with reporter genes in HEK293T cells. Chen et al. employed the parallel receptor-ome expression and screening *via* transcriptional output (PRESTO-Tango) system to identify gut microbial metabolites that regulate host physiological functions by activating GPCRs[91]. By screening hundreds of GPCRs with metabolites from over a hundred gut microbes, they revealed that numerous gut microbes generate metabolites that activate GPCRs[91]. Similarly, Colosimo et al. conducted a large-scale functional screen of molecules produced by human microbiota members and identified metabolites that interact with GPCRs associated with diverse functions in the nervous and immune systems[92]. With the advancement of HTS technology, Chen et al. further advanced this method by using next-generation sequencing (NGS)-based nucleic acid barcoding, allowing for the simultaneous evaluation of nearly all GPCRs in a single well of a 96-well plate. This significantly increased the efficiency of GPCR high-throughput screening, providing a tenfold increase in sensitivity and selectivity and a reduction in sample volume[93]. A similar approach has been applied to the HTS of ORs[60].

Researchers have argued that an over-reliance on indirect measures of GPCR activity such as the aforementioned second messengers may not precisely reflect *in vivo* effects due to the distinct signaling pathways in different cellular expression systems. To address this, fluorescence or bioluminescence-based resonance energy transfer biosensors for G-protein activity have been developed as a more direct measurement of GPCR activity. This method is also cell-line dependent and typically requires co-expression of the GPCR with the biosensor, which ensures the stability of the GPCR in the cell and is essential for the accuracy and reproducibility of the assay signal. In 2018, Mella et al.[94] introduced Nomad biosensors, a high-throughput, multiplexed GPCR assay tool that uses fluorescence to detect interactions between G proteins and second messenger transducer proteins. Upon activation by ligands, these biosensors undergo structural changes that enhance vesiculation and increase fluorescence intensity. They applied this technology to screen 1200 chemicals for endothelin B receptors, demonstrating that ET-1 activates both Ca^{2+} and cAMP signaling pathways[94]. A previous study has developed ONE-GO biosensors for various G protein types, though their application in drug screening remains to be explored[95].

4. Strategies for constructing genome-wide pan-GPCR transgenic cell library

4.1. *Limitations of current GPCR-targeted drug screening*

Many current GPCR-targeted screening methods tend to focus on a single pathway or action[96], and are restricted to a specific GPCR or a closely related subfamily of GPCRs, which may over-estimate opportunities for discovering potential side or discovering new therapeutic effects from targeting other GPCRs[97]. For instance, some current GLP-1R agonists show excellent efficacy in treating obesity, while some multiple targets exhibit even greater efficacy, like GLP-1R/glucose-dependent insulinotropic polypeptide (GIPR) dual agonist[98,99]. Moreover, the diverse GPCR family includes numerous isoforms and subunits, which present diverse and complex conformational and ligand-binding properties. These diversifications and complexities may eliminate many potential drugs in the screening, especially for isoforms with undefined structures or significant structural differences[100].

So far, many GPCRs are still oGPCRs, like some adhesion GPCRs, frizzled and taste receptors, and most ORs whose functions and ligands remain unclear[101]. This is attributed to the complex signaling pathways, lack of selective ligands, low expression levels[102,103], and the challenges of ensuring proper protein folding and PTMs in heterologous expression systems[104]. Additionally, OR expression is not confined to the nasal cavity, it also may occur in non-olfactory tissues[105]. Various strategies have been employed to enhance the surface expression of GPCRs in heterologous cells. These include introducing addition/deletion of receptor sequences[106], modifying receptor sequences[107], co-expressing chaperone proteins to facilitate proper folding[108], and using pharmacological chaperones[109]. The ORs often require special cofactors and signaling proteins for heterologous function expression[110]. The receptor-transporting protein (RTP) family, RTP1 and RTP2, as well as the receptor expression enhancer protein 1 (REEP1), facilitated the trafficking of ORs to the cell membrane and enhanced odorant-mediated responses of ORs in HEK293T cells. $G\alpha_{olf}$, acting as a signal transduction molecule, couples with ORs to activate AC, thereby increasing intracellular levels of cAMP and initiating downstream signaling pathways. Based on this, a previous study has established a HEK293T-derived stable cell line for heterologous expression ORs named Hana 3A cell line, which was made by introducing the RTP1, RTP2, REEP1, and $G\alpha_{olf}$[111]. The Hana 3A cell line has been widely utilized for OR-associated research and largely accelerated the exploration of ORs[112,113]. For instance, Jones et al. have stably expressed 39 ORs in Hana 3A cell lines to screen large chemical panels against GPCR libraries[60].

High-throughput and large-scale drug screenings still play a crucial role in identifying potential lead compounds targeting GPCRs[114]. However, the absence of known endogenous ligands for oGPCRs, makes them a challenge to study using conventional screening methods, and the undefined functionality and ligand specificity of ORs, further complicate the screening process. To date, only 314 GPCRs have been conducted for the large-scale HTS which was constructed using transient transfection[91]. The genome-wide HTS of GPCRs has not yet been reported. The future holds great promise for developing comprehensive genome-wide pan-GPCR cell line libraries, achieved through a synergistic combination of these innovative strategies. Consequently, we propose three strategies including gene overexpression, PRESTO-Tango, and CRISPR technology to construct the genome-wide pan-GPCR cell line libraries combined with various detection platforms to more thoroughly and efficiently discover new GPCR-targeted drugs.

4.2. *Construction of a genome-wide GPCR overexpression cell library*

To develop a comprehensive GPCR cell screening platform for natural compounds, emphasizing the need for cost-effective, long-term stable protein expression. We propose various methods for constructing a genome-wide GPCR overexpression cell library, using the 2nd generation of lentivirus transfection system that consists of envelope plasmid pVSV-G, the packaging plasmid pGAG-POL-REV, and the transfer plasmid[115]. The general

GPCR overexpression vector system uses one transfer plasmid overexpressing GPCR, it is also possible to choose for the co-expression of CRE-Luc for efficient detection. The OR overexpression cell line Hana 3A cell line is a transgenic HEK293T cell line that stably expresses proteins such as RTP1L, RTP2, and REEP1, which contribute to enhancing receptor expression and function[116]. Here we propose constructing the OR overexpression system using three transfer plasmids: the OR overexpression plasmid, the OR helper-pCDH that expresses RTP1S, REEP1, Ric8b, and $G\alpha_{olf}$ for facilitating the receptor expression, and the OR reporter-pCDH that expresses firefly cAMP response element binding protein luciferase (CRE-Luc) and simian virus 40 *Renilla* luciferase (SV40-RL) allowing for the efficient detection of non-olfactory GPCR activation using Luc. Cell lines containing resistance genes from the transfection vector are selected using specific antibiotics (Puro/Blast/HygR) to negative cells. A limited dilution approach is employed to screen single-clone cell lines, reducing heterogeneity and improving the phenotypic stability and consistency of the stable transgenic GPCR (Fig. 2A). Some of the genome-wide 800 GPCRs may be hard to get their transgenic cell lines through the general stable overexpression systems. In these cases, the transient transfection with HEK293T cells could be applied to make the overexpressed cells, contributing to a genome-wide pan-GPCR overexpression cell library for genome-wide drug screening.

4.3. Construction of genome-wide GPCR expression systems by applying PRESTO-Tango

PRESTO-Tango is a transcription factor activation system that relies on receptor−ligand interactions to regulate GPCR expression and activity by coupling GPCRs with transcription factors. It has emerged as a powerful tool for high-throughput functional expression and detection of GPCRs, with the ability to effectively detect over 300 GPCRs[117]. The Tango technology originated from studying the transcriptional activation domain of the tTA, which specifically binds to the tet-responsive element (TRE) and activates downstream gene expression. Barnea et al.[118] developed the Tango system based on tTA, which consists of three components: (1) the GPCR sequence with a secretory signal peptide for membrane targeting; (2) a fusion protein module combining the catalytic domains of β-arrestin and TEV protease; (3) the reporter gene module with TRE-responsive genes, typically using fluorescent or luminescent reporters such as fluorescein or RFP. Upon activation by extracellular ligands, the GPCR promotes the recruitment of repressor proteins. The repressor protein linked to

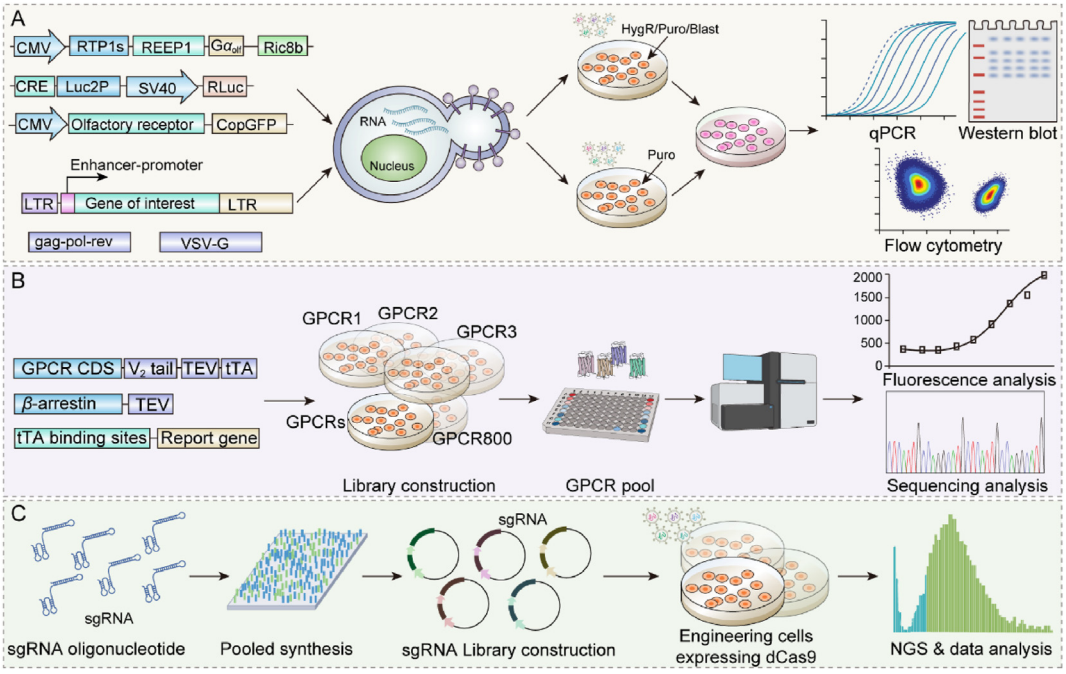

Figure 2 Strategies for the construction of genome-wide pan-GPCR stable transgenic cell libraries. (A) The genome-wide GPCR overexpression cell library is constructed by using the 2nd generation of lentivirus transfection system that consists of envelope plasmid pVSV-G, the packaging plasmid pGAG-POL-REV, and the transfer plasmid. The general GPCR overexpression vector system uses one transfer plasmid overexpressing GPCR. The OR overexpression system uses three transfer plasmids: the OR overexpression plasmid, the OR helper-pCDH that expresses RTP, REEP1, and $G\alpha_{olf}$, and the OR reporter-pCDH that expresses firefly luciferase CRE-Luc and renilla luciferase SV40-RL. The genome-wide GPCR cell library is verified using qRT-PCR, Western blotting, and flow cytometry assays. (B) The Tango system consists of three modules: a GPCR receptor plasmid, a fusion protein module encoding the catalytic domains of β-arrestin and TEV protease, and a reporter gene module, in which 800 GPCR genes are individually transfected into the target cells to converge into a GPCR cells library, and the subsequent assay is accomplished based on fluorescence observation of the reporter genes or by NGS sequencing. (C) The designed sgRNA library of 800 GPCRs was synthesized, and then the library plasmid was constructed and transfected into the target cells expressing dCas9 in the form of lentivirus. After NGS sequencing of the library cells screened by antibiotics, the genome-wide GPCR cell line library was obtained.

TRV cleaves the DNA-binding domain of tTA, enabling tTA to enter the cell nucleus and activate reporter gene expression. The activation of GPCR is dynamically monitored by fluorescent or luminescent signals[118]. Recent advancements have further enhanced the Tango system. Kroeze et al.[117] optimized the codons of GPCR sequences and incorporated sequences from the C-terminal end of the V_2 pressin receptor (V_2 tail) after each receptor sequence, expanding the Tango assay into a platform that encompasses the entire GPCRome.

The PRESTO-Tango system is an available approach for construction of a genome-wide GPCR expression library, which requires the simultaneous expression of GPCRs, fusion protein modules, and reporter gene modules in the cells. A recent study achieved the screening of 314 GPCRs using the PRESTO-Tango platform. Here, we propose to extend the attempts to genome-wide GPCR screening. PRESTO-Tango holds significant promise for applications in genome-wide GPCR high-throughput expression and screening research. Current research focuses on a single GPCR target for a specific disease, resulting in incomplete mechanistic analysis and low screening efficiency. However, by utilizing PRESTO-Tango to construct a genome-wide GPCR library, it becomes possible to simultaneously screen a large number of compounds against the entire GPCR repertoire, offering the advantages of high-throughput and cost-effective screening. In addition, further advancements in synthetic biology, microfluidic chip technology, CRISPR, DNA synthesis, and other related fields hold promise for improving the performance and practicality of the Tango assay system.

4.4. Construction of genome-wide GPCR library applying CRISPR/Cas9 technology

Tools based on CRISPR/Cas9 have developed rapidly in recent years and are widely applied in library construction[119]. To construct a genome-wide library by applying CRISPR/Cas9 technology, multiple genes may be edited in a single cell, which may lead to clear changes in the biological characteristics of cells. By evaluating these biological characteristics, genes with increased or decreased sensitivity to specific drugs can be identified. This phenotypic screening method can quickly identify potential drug targets and related drugs[120]. Currently, CRISPR/Cas9 technology finds extensive applications in library construction and GPCR research.

The CRISPR/Cas9 library is instrumental in identifying potential drug targets. Through screening the genome-wide CRISPR/Cas9 library, phosphoglycerate dehydrogenase (PHGDH) was identified as the first committed enzyme in the serine synthesis pathway (SSP) and a pivotal factor of sorafenib resistance in hepatocellular carcinoma[120]. Ligand targeting PHGDH has proven effective in overcoming tyrosine kinase inhibitor (TKI) resistance[121]. Additionally, the CRISPR library can facilitate the study of drug resistance mechanisms. Based on the genome-wide CRISPR/Cas9 gene knockout screening system, key genes associated with lovastatin resistance were identified, revealing that dual-specificity phosphatases 4 (DUSP4) deficiency mediates drug resistance through the activation of the MAPK/ERK signaling pathway. A combination therapy of lovastatin and a mitogen-activated extracellular signal-regulated kinase (MEK) inhibitor may offer a new strategy to counteract this resistance[122]. The CRISPR/Cas9 library also allows for screening the therapeutic effects of combined drugs. A double gene knockout system based on CRISPR/Cas9 generated a large-scale human genetic interaction map that identifies target pairs for synthetic lethal drugs, which exhibit a synergistic killing effect[123]. CRISPR/Cas9 library can also be used to predict the side effects of drugs[124].

CRISPR/Cas9 technology also plays a key role in the study of GPCR. Firstly, the regulatory factors of GPCR/cAMP signal transduction can be explored by using CRISPR/Cas9 technology. Semesta et al.[125] employed a whole-genome CRISPR interference (CRISPRi) screening and identified 45 strong and 50 medium phenotypic regulators associated with cAMP signal transduction. Additionally, CRISPR/Cas9 has been utilized to investigate the function of specific GPCRs. By knocking out GPR27 in 3T3 cells, researchers observed a reduction in the increase of L-lactate induced by a GPR27 agonist, suggesting that GPR27 activation enhances L-lactate production in 3T3 embryonic cells[126]. Furthermore, CRISPR/Cas9 technology aids in the identification of GPCR ligands. Using a CRISPR-constructed digital crime and cybercrime forensic investigation report (DCyFIR) yeast library, GPCR-ligand interactions or GPCR-protein interactions can be monitored in real-time, revealing dynamic changes in these interactions within cells. This approach led to the discovery that kynurenic acid not only activates GPR35 but also hydroxycarboxylic acid receptor 3 (HCAR3)[127].

The CRISPR/Cas9 technology can be applied to construct the genome-wide GPCR cell library, which could provide important resources and tools for the function, mechanism, and GPCR-targeted drug screening. The application of CRISPR/Cas9 technology to construct genome-wide GPCR libraries requires the construction of dCas9 stably transformed target cell lines with transcription activation or inhibition factor (Fig. 2C). The genome-wide sgRNAs expression can be achieved by using the 2nd lentivirus transfection system expressing a pool of sgRNAs for the 800 GPCRs in the dCas9 overexpressed cell line (Fig. 2C). The generated genome-wide GPCR gene-editing cell library can be applied for phenotype screening with potential chemicals, and the NGS can be applied to verify the edited genes in the generated individual cells (Fig. 2C). CRISPRi and CRISPR activation (CRISPRa) are two derivative forms of CRISPR/Cas9 technology that used for gene inhibition and activation, respectively. During the construction process of the genome-wide GPCR cell line library, compared with other CRISPR/Cas9 systems, CRISPRa and CRISPRi allow for partial gene inhibition or activation, rendering them more flexible and precise alternatives.

5. Remarkable potential applications of the transgenic GPCR cell library

5.1. GPCR cell library-based drug screening

Target identification and drug screening, are historically known for their time-consuming and expensive processes[128]. The introduction of virtual screening has revolutionized the screening process at every stage of new drug development[129,130]. This technology has been instrumental in the virtual screening of vast compound libraries, such as the identification of allosteric site antagonists for CCR7 from a library of 2.3 million compounds[131], and the identification of potential GPR132-targeting compounds for diabetes improvement[132]. It has also facilitated the ligands discovery within virtual libraries that contain over 11 billion compounds[133]. In the process of developing new drugs, compound libraries and GPCR databases are indispensable. Artificial intelligence (AI) driven virtual screening of compound libraries is an

essential part of innovative drug discovery and a crucial tool for HTS[134]. Here we propose a strategy of "parallel drug screening plus target screening" by integrating virtual screening with the genome-wide pan-GPCR cell libraries dependent HST platform. By screening chemical libraries with the virtual screening following genome-wide pan-GPCR cell libraries, it is possible to identify potential ligands to these well-known GPCR targets that are associated with diseases, and it also allows for the deorphanization of oGPCRs by detecting their interaction with some chemicals with known pharmacological activities. The potential drugs or new targets can be functionally characterized with further *in vitro* and *in vivo* experiments (Fig. 3). This new drug screening strategy aims to accelerate the drug discovery process, improve the efficiency and success rate of drug discovery efforts, and provide valuable insights for further drug development.

Our lab has previously developed a AI-integrated strategy for screening GPCR ligands[135,136], in which shows the numerous data produced from all kinds of omics technologies, including genomics, transcriptomics, proteomics and metabolomics, provide valuable information for GPCR drug discovery. These omics technologies have made remarkable progress in identifying GPCR targets, elucidating GPCR mechanisms[137], screening of GPCR drugs, and validating GPCR drugs[138]. For instance, phosphoproteomics technology has been used to study signaling pathway alterations within the brain triggered by GPCR agonist activation[139]. By combining the genome-wide pan-GPCR cell line libraries with multi-omics and bioinformatics technologies, we could get comprehensive profiles from differential genes, metabolites, and proteins. It allows for the generation of huge amounts of GPCR-associate omics data and promotes the birth of a novel GPCRome database, which will provide useful information for new targets and ligands discovery, GPCR signal pathway analysis, etc. offering valuable insights for GPCR drug discovery (Fig. 3).

5.2. *GPCR cell libraries facilitate the study of GPCR mechanisms*

5.2.1. *Etiology and pathogenesis*

Previous studies have employed various transgenic cell lines to investigate gene function and drug development. For example, the CRISPR/Cas9 technology has been utilized to generate adrenergic receptor-beta 2 (ADRB2) deficient HEK293T cell lines, facilitating the identification of classical $\beta 2$ adrenergic receptor ($\beta 2$AR) targets[140]. Pancreatic and lymphoma cell lines have been developed to illustrate the tumor-inhibitory effects of endogenous peptide inhibitor (EPI-X4) of C-X-C chemokine receptor type 4 (CXCR4)[141]. Colclough et al. created the MDCKII-MDR1-BCRP cell line to examine drug efflux at the blood−brain barrier[142]. Andrysiak et al.[143] established pairs of homozygous control and myotrophin-deficient human induced pluripotent stem cell lines to investigate the pathogenesis of Duchenne muscular dystrophy-associated cardiomyopathy. These studies highlight the crucial roles of cell lines in advancing our understanding of gene function and drug development.

The GPCR family is widely distributed throughout the human body and plays diverse roles in various tissues and disease states. This complexity arises from their participation in numerous biological signaling cascades[144,145]. Notably, studies have elucidated the role of specific GPCRs in different diseases. Zi et al.[146] uncovered the role of GPR39 in exacerbating biliary acute pancreatitis by establishing stable cell lines that express GPR39. GPRC6A has been identified as a potential target for controlling prostate growth and cancer progression, while GPR56 has been implicated in the adhesion signal in tumor cells[147,148]. Overexpression of GPR161 has been uncovered to be associated with triple-negative breast cancer[149].

The genome-wide pan-GPCR cell libraries, including a collection of HEK293T-derived transgenic cells that independently overexpress 800 human GPCRs and the genome-wide GPCR knockout HEK293T cell library, are a valuable resource for researching disease pathogenesis. These extensive collections will lay a solid foundation for discovering novel therapeutic targets and for a deeper understanding of GPCR function. Researchers can employ multiple tools like HTS, phenotypic analysis, multi-omics profiling, disease models, and drug screening to identify new therapeutic targets within this library and unravel the complexities of the GPCR signal (Fig. 4). Enhanced knowledge of GPCRs in physiological and pathological contexts will greatly support the development of new therapeutics and advancements in disease treatment.

5.2.2. *Post-translational modifications*

PTMs are vital in controlling various aspects of GPCRs, including their maturation[150], trafficking[151], localization[152], dimerization[153], conformational changes, ligand binding, biosynthesis, and endocytosis[154]. These modifications significantly influence signal transduction. Despite its importance, current proteomic data is insufficient to fully detail the range and nature of protein modifications in GPCRs, especially concerning their comprehensive modification profiles. Introducing a novel method that combines a genome-wide GPCR cell library with high-resolution MS can be applied to bridge this gap. This approach facilitates detailed

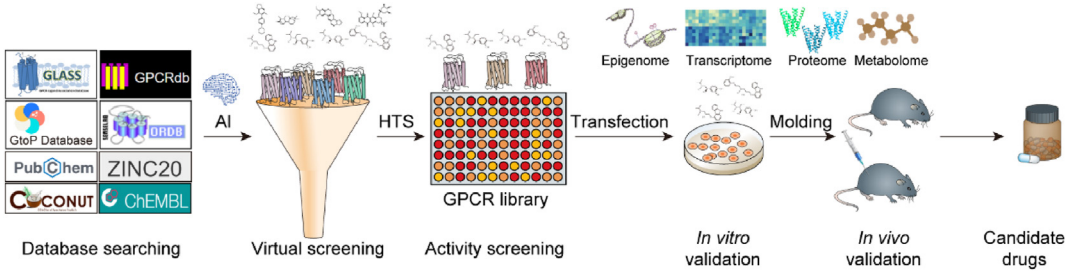

Figure 3 Workflow of applying the genome-wide pan-GPCR cell libraries in drug screening. The first step is to gather relevant data, such as GPCRs and ligands information in related databases such as GLASS (https://zhanggroup.org/GLASS/), GPCRdb (https://gpcrdb.org), GTOPdb (https://www.guidetopharmacology.org), HORDE (https://genome.weizmann.ac.il/HORDE), and Pubchem (https://pubchem.ncbi.nlm.nih.gov/). et. al. AI, artificial intelligence; HTS, high-throughput screening.

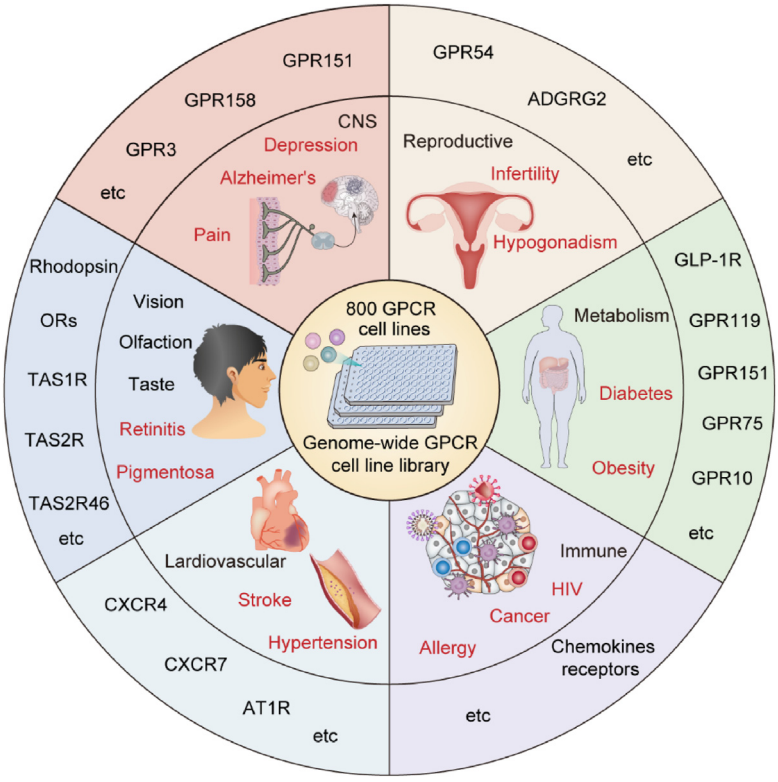

Figure 4 Overview of representative examples of human GPCRs involved in human physiological metabolism and disease development. From inside to outside are: The human genome-wide pan-GPCR cell line libraries; human physiological metabolism and major diseases (black font texts represents several major physiological systems of humans, red font texts represent some major diseases occurring in these physiological systems); Some well-studied GPCRs that have been shown closely associated with the corresponding physiological metabolism and major diseases. CNS: central nervous system. HIV: human immunodeficiency virus.

proteomic analysis of GPCR protein modifications, creating a comprehensive and high-quality GPCR modification database.

The genome-wide pan-GPCR cell libraries are invaluable, covering a broad spectrum of GPCR variants. This allows for the study of modifications across various GPCR subtypes. By employing immunoprecipitation coupled with high-resolution MS techniques, supplemented with enrichment strategies targeting specific modifications, it is feasible to identify and characterize these specific modifications, namely phosphorylation, glycosylation, acetylation, and ubiquitination, occurring within GPCR proteins, as well as predict and validate GPCR modification sites following pathological processes or pharmacotherapy using the genome-wide GPCR cell library (Fig. 5). This comprehensive analysis provides valuable insights into these modifications'

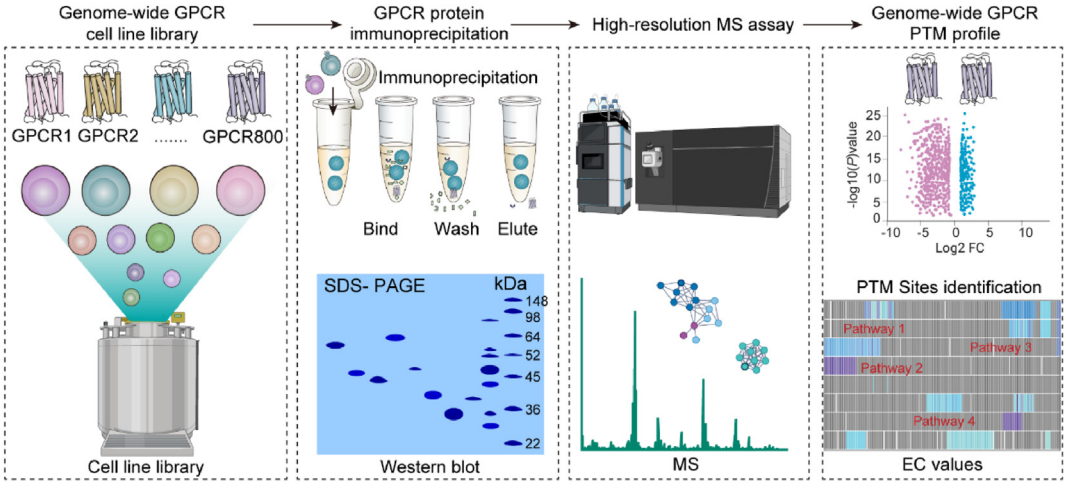

Figure 5 Experimental workflow of constructing genome-wide pan-GPCR cell libraries for comprehensive characterization of GPCR PTMs. The workflow comprises three steps: (1) GPCRs are extracted from a genome-wide pan-GPCR cell line libraries, and enriched by immunoprecipitation (acetylation, ubiquitinylation) or immobilized metal affinity chromatography (IMAC). (2) The samples are subjected to MS analysis. (3) Statistical analysis. PTM: post-translational modifications. MS: mass spectrometry.

intricate regulatory mechanisms and functional consequences. The development of a high-quality GPCR modification database will enhance our understanding of these membrane receptors. It will enable the exploration of the functional effects of specific modifications, the identification of new drug targets, and the design of innovative therapeutic approaches. Moreover, the database will support comparative analysis of modification patterns across various GPCR subtypes, helping to uncover common regulatory mechanisms and potential differences in signal pathways.

In summary, establishing a genome-wide pan-GPCR cell library and applying high-resolution mass spectrometry to explore the PTMs of genome-wide GPCRs is a powerful approach for thoroughly mapping and characterizing protein modifications within the GPCR family. This groundbreaking strategy is set to enrich our knowledge of GPCR biology and propel forward membrane receptor research.

5.3. Application of the genome-wide GPCR cell libraries in drug safety assessment

Drug safety assessment is an important issue in developing a new drug. From 1997 to 2016, eight drugs had been withdrawn from the market due to their potential to cause liver toxicity. These drugs include tolcapone, troglitazone, bromfenac, trovafloxacin, nefazodone, ximelagatran, lumiracoxib and sitaxentan[155]. Troglitazone and bromfenac were approved by the FDA in 1997, but very soon were identified as having severe liver toxicity that can be life-threatening[156,157]. Evaluating cytotoxicity and drug safety is essential in new drug research and development[158], while effectiveness is a key requirement, safety must be equally prioritized[159]. For most pharmaceutical industries, conducting safety evaluations early in the drug discovery process can minimize losses and better predict adverse reactions during later stages[160].

The commonly used experimental methods for assessing drug toxicity and safety are categorized into *in vitro* and *in vivo* experiments[161,162], like cell-based assays (*e.g.*, cytotoxicity, proliferation) and acute toxicity studies. Typically, *in vitro* methods are employed to screen and evaluate the cytotoxicity and safety of drugs[163], while *in vivo* methods are used to more comprehensively assess and simulate the toxicity and safety of drugs in whole organisms, with preclinical animal models being the most commonly used tool for drug toxicity and safety evaluation[164]. However, animal models cannot fully replicate the complexity of human diseases, and there are ethical concerns regarding animal experimentation[156]. For example, due to significant species differences in liver architecture, regenerative capacity, disease progression, inflammatory markers, metabolism rates, and drug response, translational research to model human liver diseases and drug screening platforms by animals yields limited results, leading to failure scenarios.

To overcome this impasse, over the last decade, 3D human liver *in vitro* models have been proposed as an alternative to preclinical animal models[164]. One of the methods for evaluating the cardiac safety of drugs is the toxicity assessment of single-layer myocardial cells. Zhu et al.[165] used H9c2 rat cardiomyocyte cell lines to establish and optimize a high-connotation multi-index evaluation method for traditional Chinese medicine cardiotoxicity based on cell imaging. In terms of neurotoxicity evaluation, the rational selection of various animal cells and human tumor cell lines can evaluate the non-specific cytotoxicity and specific neurotoxicity of drugs on nerve cells in terms of morphology, function, signal transduction, and other aspects from the perspective of neurotoxicity mechanism[166]. Drug safety evaluations can thus be conducted on cell lines for assessment of liver toxicity, cardiac toxicity, and neurotoxicity. Therefore, through the pan-GPCR cell library, drug safety can be evaluated based on mechanisms such as whether the drug inhibits the side effects caused by the β-arrestin signaling pathway.

Evaluating the safety of known drugs using the pan-GPCR cell lines library offers numerous benefits. The stable GPCR cell lines can provide consistent protein expression and reliability, ensuring dependable experimental outcomes[167]. Additionally, it reduces the need for animal testing, lowering costs and ethical concerns. Using pan-GPCR cell libraries for known drug safety studies is a powerful and effective method, that can provide reliable safety data for drug development and more dependable safety data for drug development and clinical applications.

6. Concluding remarks and future perspectives

The GPCR family encompasses numerous members with diverse functions, offering significant potential for new target and ligand drug discovery[168]. To facilitate drug discovery and GPCR research, it is essential to establish appropriate cell expression systems through various biotechnological methods. Large-scale cell-based screening has emerged as a valuable tool, greatly enhancing the efficiency and precision of drug development. Here we advocate the construction of genome-wide GPCR cell libraries by applying the gene overexpression, CRISPR, and PRESTO Tango, following various analyses for different experimental purposes, for accelerating GPCR-targeted drug screening These genome-wide cell libraries will not only empower drug research and development but also will provide abundant resources for fundamental GPCR biology research.

Identifying lead compounds is pivotal in contemporary drug development[169]. HTS[170], fragment-based and structure-based drug design (FBDD/SBDD)[171], and DNA-encoded library (DEL) technologies form the cornerstone for innovative drug development through original research[172]. Expanding on these technologies, a large-scale natural drug screening method that integrates AI with genome-wide pan-GPCR cell libraries and compound libraries has been proposed (Fig. 3). Leveraging AI and muti-omics data[14], it aims to refine the structures of identified active ligands and assess their biological activities. This approach promises to bring new insights into future drug development and traditional drug target research, streamlining the drug discovery process and paving the way for more efficient and effective therapeutics.

However, the genome-wide pan-GPCR cell libraries also have some limitations. First, the individual transgenic cells may present unequal GPCR expression levels in the genome-wide GPCR cell libraries, which may affect the accuracy of the screening results. Moreover, GPCRs heterologously expressed in HEK293T or Hana 3A cells are impossible to get similar expression levels as in our human bodies, and may even undergo different PTMs, which are not able to fully simulate these internal environments[173]. Therefore, the cell libraries are mainly applied for large-scale ligands or target screening, and further *in vitro* and *in vivo* functional characterization are always needed for the final verification. We may get rid of some real targets or ligands during the screening due to the potential divergence of GPCR expression and PTMs between the cell libraries and human tissues. However, it's still possible to

obtain some of the potential targets or ligands when screening such a large-scale cell library with large chemical compound libraries. More explorations are required to achieve a more efficient cell screening. Many advancements in technology may provide promising solutions to overcome these limitations[174]. Integrating innovative approaches such as 3D cell culture models[175], microfluidic platforms[176], live-cell imaging techniques[177,178], and other cutting-edge technologies like muti-omics holds the potential to address these challenges. This comprehensive integration aims to surpass the current limitations of genome-wide cell screening systems, enhancing their accuracy, physiological relevance, and predictive capabilities. By adopting a holistic and interdisciplinary approach, we can pave the way for more precise, efficient, and targeted drug discovery processes in GPCR research and functional genomics.

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Author contributions

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Conflicts of interest

The authors declare that they have no conflict of interest.

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