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A REVIEW ON CURRENT AND FUTURE CHALLENGES IN GPCR DRUG DISCOVERY

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Abstract
G-protein-coupled receptors (GPCRs) are essential components in cell signaling, influencing a wide array of physiological processes. They are the largest and most diverse family of cell surface receptors, and over the years, significant progress has been made in understanding their structure and function. Since the cloning of the first GPCR in 1986, research has unveiled their critical role as drug targets, leading to their status as the most successful receptor class for approved therapeutic agents. In drug discovery, insights into receptor dynamics-ranging from agonist to antagonist effects—are fundamental to developing effective therapies. Advances in structural biology and molecular simulations have provided a deeper understanding of GPCR activation mechanisms, which are essential in designing drugs that can either enhance or inhibit receptor activity. Allosteric sites, which provide alternative pathways for drug modulation, are emerging as promising targets for future drug design. Recent studies have focused on small-molecule allosteric modulators, revealing how they interact with receptors at the molecular level to produce therapeutic outcomes. The growing knowledge of GPCR mechanisms is poised to advance the development of innovative therapies, with new technologies and methodologies enabling the creation of more selective and effective drugs. Future research will continue to explore GPCRs' complex signaling networks, providing critical insights into their potential for treating a variety of conditions.

Keywords: G-Protein Coupled Receptors (GPCR), Signature discovery, Drug discovery Small molecule Biomarker Allosteric drug; biased drug, bitopic ligand; bivalent ligand, heteromer-selective drug.

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Introduction
GPCRs are the largest superfamily of cell surface transmembrane receptors, with 821 human genes. Approximately 34% of marketed drugs are modulators of GPCR function. GPCRs are involved in various physiological processes, making them attractive targets for drug discovery. Despite their importance, the natural ligands and functions of approximately 140 GPCRs remain unknown. GPCR drugs appear in nearly all therapeutic areas, especially neurology, cardiovascular, and inflammation. There is less GPCR drug presence in oncology, but this will increase with targeting immune cells [1]. Orthosteric ligands impose an effective alteration on GPCR activity and signaling process. However, subtype selectivity remains an intractable issue due to the sequence conservation of orthosteric sites. Targeting allosteric sites alone or targeting both orthosteric and allosteric sites can overcome these major hurdles. Advances in technologies like crystallography, cryo-EM, and NMR have improved our understanding of GPCR biology. GPCRs exhibit a versatile seven-transmembrane framework that acco

mmodates receptor activation with various ligand sizes. The activation of GPCRs is mediated downstream by many integrated biological processes and linked to diverse cellular functions. Alterations in protein structure can be associated with decreased drug therapeutic efficacy and patient-related side effects[2]. GPCRs constitute the most important superfamily of membrane proteins pharmacologically targeted in clinical practice. Approximately 35% of FDA-approved drugs target GPCRs. The development of GPCR-targeting drugs has been achieved using classical and modern techniques and methodologies. These drugs activate, block, or modulate GPCR function by targeting orthosteric or allosteric centers. GPCR MD studies have provided insights into GPCR signaling processes and the structural basis for small-molecule drug design [3].

Emerging opportunities and prospects

Breakthroughs in GPCR biology have generated vast amounts of data, enabling innovative drug design. Artificial intelligence will refine this data, guiding the discovery of effective therapeutic agents. Future prospects hold promise for transformative advancements, including de-orphanization of orphan GPCRs and harnessing polypharmacology. AI-driven data analysis will uncover innovative treatments for various diseases, streamlining the process of identifying promising lead compounds [4].

Experimental screening in GPCR targeted drug discovery

Structure-based drug design is a valuable tool for identifying potential therapeutic compounds. However, in silico methods have limitations, requiring experimental confirmation to validate efficacy. Experimental screenings involve binding assays or biophysical screening methods to determine compound affinity. Orthosteric drugs account for over 50% of FDA-approved GPCR drugs, but development is stagnant. Allosteric modulators offer a promising alternative, allowing for nuanced GPCR signaling and reduced side effects. Experimental validation is crucial to confirm efficacy and optimize compound design [5].

Novel screening technology

GPCRs are a prominent family of therapeutic targets. Various experimental technologies have been developed to screen for GPCR-targeted ligands, including binding-based, stability-based, and cell signalling-based assays. DNA-encoded library (DEL) and affinity selection MS have been adapted to GPCR ligand discovery. DEL enables screening of billions of small molecule-DNA conjugates, leading to novel ligand discoveries. Affinity selection MS detects ligands physically associated with a GPCR, facilitating identification of orthosteric and allosteric modulators. Membrane-based affinity MS is a powerful approach for ligand screening toward wild-type active GPCRs [6].

- **Source**

Novel screening technologies, such as DEL and affinity selection MS, have revolutionized GPCR drug discovery, enabling unbiased ligand detection and accelerating therapeutic agent discovery [7].

Structure-based drug design

Structure-based drug design (SBDD) has emerged as a powerful approach to discovering novel therapeutic agents targeting GPCRs. SBDD exploits structural information of protein targets and knowledge of known ligands to identify potential lead compounds. Recent advances in SBDD have been driven by the increasing availability of high-quality GPCR structures, enabling researchers to develop more accurate computational models and docking algorithms. SBDD has been successfully applied to various GPCRs, including dopamine receptors, to identify potent and selective antagonists. It has also been used to design subtype-selective compounds and identify novel binding sites and allosteric modulators. The use of SBDD in GPCR drug discovery has several advantages, including improved accuracy and efficiency. Recent studies have demonstrated the power of SBDD in discovering novel GPCR modulators, including potent and selective agonists for the β2-adrenergic receptor and subtype-selective antagonists for the muscarinic acetylcholine receptor. The increasing availability of high-quality GPCR structures has enabled researchers to develop more accurate computational models and docking algorithms, improving the accuracy of SBDD predictions [8].

Molecular mechanisms of ligand binding to GPCRs

Understanding ligand recognition by GPCRs is crucial for pharmaceutical research. Enhanced molecular dynamics (MD) approaches, such as random acceleration MD (RAMD) and steered MD (SMD), have been developed to simulate ligand binding. These methods have revealed ligand exit pathways and binding mechanisms for various GPCRs, including β2-adrenergic receptor (β2AR) and muscarinic acetylcholine receptors (mAChRs). Specialized supercomputers like Anton have enabled conventional MD simulations, providing insights into ligand binding pathways. Accelerated MD (aMD) and metadynamics simulations have also been used to study ligand binding, capturing binding mechanisms and providing valuable insights into GPCR-ligand interactions. These studies are crucial for developing novel therapeutic agents targeting GPCRs [9].

GPCR oligomerization and drug

GPCRs can form oligomeric entities, including homomers and heteromers, which have distinct properties and functions. These complexes have been detected in native tissues and can modulate each other's function through allosteric interactions. Oligomerization is a dynamic process favored by the 7-TM helix structure of GPCRs. Allosteric interactions within GPCR oligomers can occur in various ways, leading to the appearance of new allosteric centers. Understanding GPCR oligomerization and allosteric interactions is crucial for developing novel therapeutic strategies. Further research is needed to explore the therapeutic potential of GPCR oligomers and to elucidate their structure, function, and regulation. Targeting GPCR oligomers may offer new opportunities for therapeutic intervention [10].

Conformational sampling of GPCR deactivation and activation pathways

Recent advances in X-ray crystallography and molecular dynamics (MD) simulations have provided valuable insights into GPCR activation mechanisms. MD simulations have revealed distinct conformational states and dynamics in GPCRs bound by different ligands. For example, full

agonists select active and inactive states, while inverse agonists select only inactive states. Metadynamics and accelerated MD simulations have identified important low-energy intermediate states and large-scale structural rearrangements during activation.[11] The activation of GPCRs involves complex interactions between the receptor, ligands, and lipid membrane. MD simulations have provided a detailed understanding of these interactions and their role in receptor activation. Markov state models have been used to analyze simulation data, providing a detailed understanding of activation mechanisms. These simulations have revealed the importance of specific interactions between ligands and receptors, and have provided valuable insights into GPCR activation mechanisms [12].

GPCR pharmacology

The explosion of 3D GPCR structures and computational simulations has revealed the dynamic conformations of GPCRs and their role in receptor activation. The flexibility of the receptor binding pocket allows for complex pharmacological mechanisms, including biased signaling, allosteric modulation, and polypharmacology.[13] Polypharmacology, the ability of a single ligand to bind to multiple targets, has emerged as a promising approach to treating complex diseases. This approach has been successfully applied to the development of therapeutic agents targeting GPCRs, including β2AR, DRD2, and AGTR1. Biased agonism, the ability of a ligand to selectively activate specific signaling pathways, has also been explored as a therapeutic strategy. Biased agonists have been developed for several GPCRs, including μ-OR, κ-OR, and β-adrenergic receptors [14].

These advances have enabled researchers to identify and characterize allosteric binding sites on GPCRs. Allosteric modulators have been developed for several GPCRs, including GLP-1R, acetylcholine receptors, and cannabinoid receptors. These modulators have shown promise in preclinical studies, and some have advanced to clinical trials. The use of allosteric modulators has several advantages, including improved selectivity and reduced side effects. Allosteric modulators can also be used to target highly conserved orthosteric sites, which can be challenging to target with traditional orthosteric ligands [15].

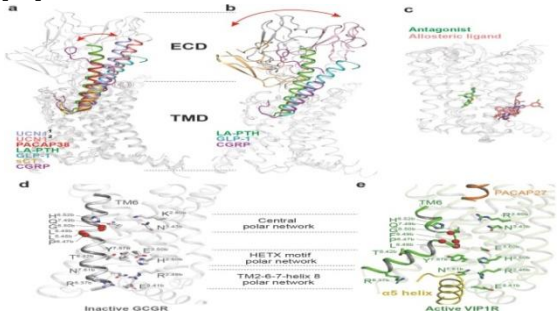

Fig. 1 Structural features and common activation mechanism of class B GPCRs. a, b Structural features of the peptide-binding pocket. The shift of peptide C-terminus (a) and ECD (b) is indicated as red arrows. The peptides urocortin 1 (UCN1)1 bound to CRF1R (light blue, PDB code: 6PB0), UCN12 bound to CRF2R (salmon, PDB code: 6PB1), PACAP38 (red, PDB code: 6P9Y), long-acting PTH (LA-PTH, green, PDB code: 6NBF), GLP-1 (cyan, PDB code: 5VAI), sCT (yellow, PDB code: 6NIY), and CGRP (magenta, PDB code: 6PB1) are shown as cartoons. Binding poses of the antagonist (green) and allosteric ligand (salmon) are shown as sticks (c, PDB codes: 4K5Y, 5EE7, 4Z9G, 5VEW, and 5VEX). d, e The common activation mechanism of class B GPCRs as exampled by the structures of inactive GCGR (gray, PDB code 3NYA) and active VIP1R (green, PDB code 6VN7). Side chains of residues in three conserved polar network are shown in stick presentation. The conserved P6.47bxxG6.50b motifs in TM6 are shown as single red spheres.

Functional diversity of GPCR

GPCRs play a crucial role in regulating various physiological processes and are categorized into several subfamilies based on structural and functional characteristics. Class A GPCRs are the largest and most extensively researched subfamily, involved in processes such as cardiovascular function, neurotransmission, and sensory perception. Other subfamilies include Class B (secretin and adhesion receptors), Class C (glutamate receptors), and Class F (frizzled receptors). GPCR signaling networks can be complex, leading to low selectivity and potential adverse effects [16]. Mutations in GPCRs can lead to various human diseases, including inherited monogenic diseases and cancer. Therapeutic approaches to GPCR pathologies include symptomatic and etiological treatment, pharmacological chaperones, and gene editing techniques. GPCRs are activated by extracellular ligands, which bind to orthosteric or allosteric sites, regulating various cell signaling pathways [17]. MD simulations have advanced our understanding of GPCR dynamics and functional mechanisms, but further research is needed to clarify aspects such as cooperative binding, allosteric modulation, and interactions with intracellular proteins [18]. A detailed understanding of GPCR molecular mechanisms is crucial for developing effective therapeutic strategies targeting these receptors. GPCRs are dynamic molecules that undergo significant conformational changes upon ligand binding, activating various signaling pathways [19]. The complexity of GPCR signaling networks requires a comprehensive understanding of their structure, function, and regulation. Recent advances in structural biology, computational modeling, and biophysical techniques have provided valuable insights into GPCR function. However, further research is needed to elucidate the molecular mechanisms underlying GPCR signaling, including the role of allosteric modulators, biased agonism, and receptor oligomerization [20].

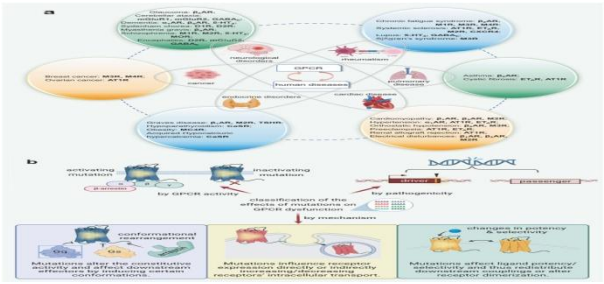

Fig 2: A) Categories of Representative human diseases caused by GPCR dysfunctions.
B) Classification of the effects of mutations on GPCR dysfunctions

Conclusion

Recent advances in crystallographic, biochemical, and computational studies have provided significant insights into the regulation of GPCR structures. To date, 388 orthosteric modulators and 717 complex structures of GPCRs bound to orthosteric modulators have been reported. In addition, 53 allosteric small-molecule modulators bound to GPCR complex structures and nine different allosteric sites have been solved. Understanding GPCR structures and mechanisms is crucial for developing effective therapeutics. Orthosteric modulators compete with endogenous ligands, and representative cases of orthosteric drugs have been analyzed, including μ-iloperidone, S1PR-siponimod, and OX2R-lemborexant complexes. Structural analysis revealed that modulators are stabilized within the orthosteric pockets by key polar interactions with residues on the TM bundles. The extracellular vestibule inside 7TMD has been identified as the most prevalent binding site for allosteric modulators. Allosteric modulators can alter the free-energy landscape and stabilize different dominant conformations of the receptor. A novel classification of allosteric modulators into two categories has been established, based on their ability to modulate orthosteric ligand binding or intracellular transducer protein binding.

Author contributions

All authors are contributed equally.

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Declaration competing interest

The authors have no conflicts of interest to declare.

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