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Conceptual Progress for the Improvements in the Selectivity and Efficacy of G Protein-Coupled Receptor Therapeutics: An Overview

Kyeong-Man Kim*

College of Pharmacy, Chonnam National University, Gwangju 61186, Republic of Korea

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G protein-coupled receptors (GPCRs) constitute a family of membrane proteins that transduce exterior stimuli into intracellular signals. GPCRs are also known as seven-transmembrane domain receptors, heptahelical receptors, serpentine receptors, or G protein-linked receptors (Lefkowitz, 2000).

In the 1970s and early 1980s, major biochemical and pharmacological GPCR studies were coincidently conducted for the apparently unrelated visual and hormonal signaling pathways of rhodopsin and β_2 adrenergic receptor (β_2AR). With the advent of molecular biological techniques, massive numbers of nucleotide sequences coding for these proteins were determined. The decade beginning in the mid-1980s was a golden age for gene cloning; a number of membrane receptors were also cloned (Nathans and Hogness, 1983; Dixon *et al*., 1986). Hydropathicity analyses of cloned proteins revealed that rhodopsin and β_2AR shared a seven transmembrane domain topology. This unexpected finding was the first indicator that heptahelical domains are a structural signature of membrane receptors that mediate signal transduction on the plasma membrane. Thereafter, ~800 GPCRs have been identified and linked to a broad spectrum of physiological processes. These processes include neurological, cardiovascular, and endocrine functions, as well as vision, taste, smell, and pain (Bjarnadottir *et al*., 2006).

β_2AR was originally recognized as a regulatory component that controls the activity of adenylyl cyclase (Robison *et al*., 1967). Progress in β_2AR research was made by pharmacological characterization using radioligands and affinity purification of β_2ARs (Lefkowitz and Haber, 1971; O'Hara and Lefkowitz, 1974). Around the same time, the research group of Alfred Gilman proposed the presence of a common protein component linking the receptors and adenylyl cyclase (Rodbell *et al*., 1971). This protein was later revealed to be Gs, a heterotrimeric GTP-binding protein (Cassel and Selinger, 1976). Subsequently, a series of G-family proteins were identified (Gilman, 1987). The main reasons why visual rhodopsin and β_2AR were selected for the GPCR studies were technical. Rhodopsin is abundant in the rod outer segment (Stryer, 1986). In the case of β_2AR, various β-adrenergic ligands were available, and they were readily amenable to radiolabelling and affinity chromatography.

Recent progress in the crystallographic structure determination of GPCRs (Cherezov *et al*., 2007; Rosenbaum *et al*., 2007) has enhanced the knowledge of GPCR structure, from 2D to 3D, and is expected to enable the design of specifically targeted therapeutics. The long-held belief that GPCRs signal exclusively through G proteins no longer seems accurate. Previously unexpected roles of some proteins, such as β-arrestins as mediators of G protein-independent signaling, led to the concept of biased signal transduction of GPCRs (Wei *et al*., 2003; Reiter *et al*., 2012). Subsequent findings of ligands that selectively activate one of more than two available signaling pathways confirmed this unprecedented phenomenon of biased agonism (Violin and Lefkowitz, 2007; Whalen *et al*., 2011; Weichert *et al*., 2015). The discovery of biased signaling of GPCRs helped break the myopic classical view that GPCRs signal exclusively through G proteins even though the molecular basis of coupling of GPCRs with different intracellular effectors remain unclear. With more high-resolution structural information of GPCRs in complex with different intracellular effector proteins such as G proteins or β-arrestins will unlock the possibility for development of therapeutics with enhanced selectivity and efficacy.

Along with molecular and structural biological research, studies with knockout animals or ones with hypomorphic mutations are increasingly impactful. In connection with complet-

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***Corresponding Author**
E-mail: kmkim@jnu.ac.kr
Tel: +82-62-530-2936, Fax: +82-62-530-2949

ed human genome projects, a genomic approach will be important to understand the link between GPCR polymorphism and heterogeneity of drug efficacy in the population.

This special issue of *Biomolecules and Therapeutics* highlights the current progress in GPCR basic research. We explore structure-based conformational dynamics of G proteins and GPCRs during activation processes, G protein- and β-arrestin-biased signaling of GPCRs, regulation of GPCR endocytosis, and evolutionary concepts for discovery and functional identification of GPCR gene families. In addition, the emerging roles of GPCRs in the pathogeneses of various diseases are reviewed; overstimulation of β_2AR in the development of insulin resistance, and viral hijacking of G protein-coupled receptors are discussed as examples. Finally, novel strategies for the design of drugs with increased specificity and reduced adverse effects are discussed. These strategies include exploitation of the potential advantages of the pharmacological feature of biased signaling, introduction of evolutionary comparative analysis, normalization of virally-dysregulated host GPCR signaling, and ligands of sphingosine-1-phosphate. The latter exemplifies drug discovery through investigation of interactions between GPCRs and its partners.

In response to agonist stimulation, GPCRs couple to the GDP-bound form of G proteins, and GDP on the Gα subunit is replaced by GTP, which induces dissociation of the Gα subunit from the GPCR and Gβγ subunits. In the first review, "Recent progress in understanding the conformational mechanism of heterotrimeric G protein activation," Duc *et al*. (2017) discuss the conformational dynamics of G proteins and GPCRs during activation processes. They introduce biochemical and biophysical results that were used to determine critical regions in the Gα subunit or GPCR responsible for the selective binding. They focus on molecular details involved in GPCR-mediated allosteric conformational changes of G proteins, as well as G protein-mediated allosteric modulation of GPCRs. Ligand-induced conformational changes in GPCRs that allow recognition of specific cognate G proteins and structural mechanisms involved in the GPCR-mediated arrestin activation process are suggested as research topics for the near future.

As their names imply, arrestins have been chiefly considered to be mediators that induce GPCR desensitization via steric hindrance. However, over the last decade, β-arrestins are increasingly being recognized as mediators of G protein-independent signaling (Reiter *et al*., 2012). In the second review, "Biased G protein-coupled receptor signaling: New player in modulating physiology and pathology", Bologna *et al*. (2017) focus on the recent progress in biased signaling of GPCRs through β-arrestin. They also discuss the effects of biased ligands on disease pathogenesis and regulation. Due to increased specificity and reduced adverse effects, biased ligands may have therapeutic potential. It is proposed that ligands that induce G protein-biased signaling stabilize GPCRs at conformations distinct from those induced by β-arrestin-biased ligands. As Duc *et al*. (2017) suggest in the first review of this special issue, upgraded structural information is required to unravel the principles of the ligand-dependent stabilization of GPCRs at conformations suitable for coupling to G proteins and β-arrestins. This review also discusses biased ligands for the β-adrenergic receptor, angiotensin II type I receptor, apelin receptor, histamine receptor, dopamine receptor, opioid receptors, and cancer-related receptors (endothelin-1 receptor, CXC chemokine receptor 4, and protease activated receptor 2).

Along with signaling through G proteins, agonist-induced conformational changes in the intracellular domains promote the association of the receptor with G protein-coupled receptor kinases (GRKs) (Zheng *et al*., 2016). Most GRK2/3 subsequently phosphorylate the specific serine/threonine residues located within the intracellular domains of the activated receptor. Next, β-arrestins located in the cytosol are recruited to activated receptors, and by interacting with endocytic machinery such as clathrin and the adaptor protein 2 complex, the β-arrestins target the GPCRs for endocytosis. In the third review, "Multifactorial regulation of G protein-coupled receptor endocytosis," Zhang and Kim (2017) discuss factors influencing the endocytosis of GPCRs. This review focuses on (i) functional interactions between homologous and heterologous pathways, (ii) methodologies for determining receptor endocytosis, (iii) experimental tools for determining specific endocytic routes, (iv) roles of small guanosine triphosphate-binding proteins in GPCR endocytosis, and (v) roles of post-translational modification in GPCR endocytosis.

The phenomenon in which the signaling of a given receptor is regulated by a different class of receptor is designated as transactivation or crosstalk. Receptor tyrosine kinases (RTKs) and GPCRs are two major membrane receptor groups that employ distinct signal transduction systems. It has long been known that agonists of some GPCRs can activate RTKs in the absence of growth factor stimulation (van Biesen *et al*., 1995; Daub *et al*., 1996). These observations have led to the concept of "transactivation" or "crosstalk," which describes a phenomenon in which a given receptor is activated by a ligand of a different class of receptors. Reciprocally, some studies indicate that RTK ligands themselves can trans-regulate GPCRs (Chou *et al*., 1998; Chen *et al*., 2008). In the fourth review, "β-Adrenergic receptor and insulin resistance in the heart", Mangmool *et al*. (2017) discuss the relationship between β-adrenergic receptor activation and insulin resistance. The authors propose that sustained overstimulation of β_2ARs enhances insulin resistance in the heart by inhibiting glucose transporter 4-mediated glucose uptake. In addition, they propose that β-blockers or inhibition of GRK2 may remediate insulin resistance in the heart.

High-throughput screening is an experimental approach employed in modern drug discovery. This technique has the advantage of rapid and efficient handling of a large number of samples (Agresti *et al*., 2010). Furthermore, novel strategies for sophisticated and target-oriented drug design are being developed. As discussed by Duc *et al*. (2017) in the first review, the availability of high-resolution crystal structures of GPCRs enabled rational and targeted drug design. In the fifth review, "Evolutionary and comparative genomics to drive rational drug design, with particular focus on neuropeptide seven-transmembrane receptors," Furlong and Seong (2017) introduce another aspect of bioinformatics-based drug design. They discuss the evolution of GPCR neuropeptide receptor gene families in vertebrate genomes. They also introduce evolutionary concepts and comparative analysis techniques used in gene discovery, gene function identification, and novel drug design.

Viruses use diverse approaches to take advantage of host cells for successful replication and continued pathogenesis. Hijacking host GPCRs into the viral genome is a tactic commonly deployed by viruses (Sodhi *et al*., 2004). Virally encoded GPCRs (vGPCRs) are close evolutionary relatives of some

host chemokine receptors. In the sixth review, "US28, a virally-encoded GPCR as an antiviral target for HCMV infection", Lee *et al*. (2017) discuss US28 of human cytomegalovirus (HCMV) as the best-studied example of vGPCRs to manipulate the signaling of host GPCRs. The authors discuss various aspects of US28, a representative GPCR of HCMV, which includes host signaling pathways hijacked by US28, endocytic properties of US28, structural and functional properties of US28, roles of US28 in the life span and pathogenesis of HCMV, roles of US28 in HCMV-mediated oncogenesis, and various regulatory ligands and modulators of US28.

GPCRs represent a major drug target in all clinical areas. According to the fifth review (Furlong and Seong, 2017) and the seventh review (Park and Im, 2017), GPCRs are the largest membrane-bound receptor superfamily in humans, with over 840 members. Because of the variety and selectivity of GPCRs, approximately 30-40% of current prescription drugs target them. In the last review of this issue, "Sphingosine-1-phosphate receptor modulators and drug discovery," Park and Im (2017) discuss the status of drugs targeting sphingosine-1-phosphate receptors, with a focus on potential clinical applications.

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