Karolinska Institutet

Department of Physiology & Pharmacology

FRIZZLED AS A G PROTEIN-COUPLED RECEPTOR

The role of Disheveled and G proteins for signal specification

AKADEMISK AVHANDLING
som för avläggande av medicine doktorsexamen vid Karolinska Institutet offentligen försvaras i Farmakologens föreläsningssal, Nanna Svartz väg 2, 171 77 Stockholm

Fredagen den 6 september 2013, kl 9.00

av
Michaela B. C. Kilander
M. Sc.

Huvudhandledare:
Docent Gunnar Schulte
Karolinska Institutet
Department of Physiology & Pharmacology
Section of Receptor biology & signaling

Bihandledare:
Professor Rune Toftgård
Karolinska Institutet
Department of Bioscience and Nutrition
Center for Biosciences

Doctor Vitězslav Bryja
Masaryk University
Department of Cytokinetics
Institute of Experimental Biology

Fakultetsopponent:
Associate Professor Stephane Angers
University of Toronto
Leslie Dan Faculty of Pharmacy

Betygsnämnd:
Professor Nico Dantuma
Karolinska Institutet
Department of Cell and Molecular Biology (CMB)

Professor Tommy Andersson
Lunds Universitet
Department of Laboratory Medicine
Cell and Experimental Pathology

Professor Barbara Cannon
Stockholms Universitet
The Wenner-Gren Institute
Physiology

Stockholm 2013

ABSTRACT

Cell communication governed and coordinated by the WNT family of lipoglycoproteins comprises essential physiological processes active throughout the life-span of metazoan organisms. In humans, severe diseases and congenital birth defects have been directly linked to disturbances in WNT signaling. In line with the general scheme of cell signaling transduction, WNT proteins bind and activate cell surface receptors in order to relay signaling to intracellular effector molecules which subsequently acts to execute changes in cell fates; such as proliferation, differentiation, migration and polarization. Thus far, a variety of structurally and functionally different proteins are identified as WNT receptors and/or co-receptors.

The ten members of the Frizzled (FZD) family of seven transmembrane-spanning proteins are the preponderant receptors of WNTs and are shown to interact with heterotrimeric G proteins and the scaffold phosphoprotein Dishevelled (DVL) to activate downstream signaling events. Due to homology in secondary structure, FZDs are classified as G protein coupled receptors (GPCRs), but display unconventional constitution and signaling properties in respect to e.g. the well-studied Class A 7TMRs. This fact generated the generally accepted notion that FZDs in fact are not GPCRs. The work presented in this thesis investigates the functional and molecular relationship between WNTs, FZDs, G proteins and DVL from a pharmacological point of view.

The link between WNTs and G proteins has been established mainly through epigenetic studies and in genetically manipulated systems. Using the [γ-^{35}S]GTP method to assay G protein activity we demonstrated that WNTs are able to activate G proteins of the PTX-sensitive $G_{i/o}$ family even at endogenous protein levels, thus providing evidence that the WNT-G protein connection indeed is not an artifact imposed by alterations in protein stochiometry.

Functional selectivity or biased agonism is a recently established feature of ligand activity at GPCRs and have provided important insights into the pharmacological aspects of 7TMR signaling. By assessment of downstream WNT signaling events and FRAP analysis of FZD_6 lateral diffusion we find that WNT isoforms are not homologous in signaling pathway activation at a specific receptor or in a defined cellular milieu. Thus, our conclusion suggests that WNTs might be able to act as endogenous biased agonists at FZDs.

Even though recent biochemical and bioinformatics data provides the definite evidence of FZDs as true GPCRs, signal transduction mechanisms and selectivity in the interactions of FZDs, G proteins and DVL are largely unknown. Here we report, using a double fluorophores FRAP and cell surface cross-linking approach, that FZD_6 protein precouples to $G\alpha_{i1}$ and $G\alpha_q$ and that the interaction is sensitive and dynamic to WNT stimulation. We also demonstrate that DVL is an essential component in the FZD_6-G protein precoupled complex. Interestingly, we find that the effect of DVL is concentration-dependent: a low as well as a high concentration of DVL destabilizes the receptor-G protein complex. Additionally, we establish that a point mutation, R511C, in the FZD_6 C-terminal region gives rise to autosomal recessive nail dysplasia and renders the receptor dysfunctional in G protein precoupling.

ISBN 978-91-7549-256-8