Abstract

Adenylyl cyclases (ACs) are a family of enzymes that catalyze the conversion of ATP into secondary messenger, cAMP that can go on to do further downstream signaling events. AC5 is one of the AC family membrane-bound members[1]. AC5 knock-out and inhibition showed improvement in cardiovascular diseases[2,3]. AC5 is regulated by both $G\alpha_s$ and $G\alpha_i$, which are Gα stimulatory and Gα inhibitory subunits respectively[4]. We know a lot about general AC-$G\alpha_s$ complex[5], but we don't have much information about the AC5 regulation mechanisms by $G\alpha_i$. Moreover, there has not been any resolved structure for full-length AC5. As a matter of fact, the only AC that has a full-length resolved structure is AC9, which was only published in 2019[6].

The aim of this project is to obtain a structure of AC5-$G\alpha_i$ complex in order to understand the AC5 regulation mechanisms, as well as conformational changes that differentiate between the active vs inactive forms of AC5. We purified $G\alpha_i$ myristoylated and unmyristoylated, and performed a co-immunoprecipitation with AC5. We showed inhibition of AC5 by both entities if they are GTP-activated compared to GDP-bound myristoylated $G\alpha_i$. We have also tried to perform cryo-EM analysis for the obtained complex, but the grids showed protein aggregates, and a few particles that are not enough for obtaining a high-resolution map.

1- Sunahara, R.K. and Taussig, R. (2002) Isoforms of mammalian adenylyl cyclase: multiplicities of signalling. Mol. Interv. 2, 168–184
2- Lai L, Yan L, Gao S, et al. Type 5 adenylyl cyclase increases oxidative stress by transcriptional regulation of manganese superoxide dismutase via the SIRT1/FoxO3a pathway. Circulation. 2013;127(16):1692-1701.
3- Okumura S, Vatner DE, Kurotani R, et al. Disruption of type 5 adenylyl cyclase enhances desensitization of cyclic adenosine monophosphate signal and increases Akt signal with chronic catecholamine stress. Circulation. 2007;116(16):1776-1783.
4- Sadana R, Dessauer CW. Physiological roles for G protein-regulated adenylyl cyclase isoforms: insights from knockout and overexpression studies. Neurosignals. 2009;17(1):5-22.
5- Tesmer JJ, Sunahara RK, Gilman AG, Sprang SR. Crystal structure of the catalytic domains of adenylyl cyclase in a complex with Gsalpha.GTPgammaS. Science. 1997;278(5345):1907-1916.
6- Qi C, Sorrentino S, Medalia O, Korkhov VM. The structure of a membrane adenylyl cyclase bound to an activated stimulatory G protein. Science. 2019;364(6438):389-394.