Discovery of surrogate ligands for a GPCR based on the 3D receptor model.

Takeshi Hiramoto

G-protein coupled receptors (GPCRs) play crucial roles in many physiological functions in the whole human body, so that GPCRs represent one of the most critical classes of confirmed drug targets. The elucidation of the 3D structures of GPCRs is indispensable for novel drug design. However, since a membrane bound protein such as a GPCR is not easily crystallized, an X-ray crystallography analysis cannot be expected. Therefore, I have conducted *in silico* and *in vitro* studies to elucidate a 3D structure of a ligand-docked GPCR and to develop a new biorational approach for the drug discovery using the 3D structure.

First, I constructed the 3D structure of bovine rhodopsin by using Fourier transform analysis and a homology modeling. I found that the 3D model showed a good agreement with the reported retinal binding (Section I). Using the similar method, I constructed the model structure of the human $P2Y_1$ receptor, one of the GPCRs. Then, I made the docking model of ADP and hP2Y1 receptor complex. The model showed that ADP reasonably bound inside the helical bundle known to an agonist and antagonist binding sites. Then I employed an *in silico* screening for endogenous compounds to select possible $hP2Y_1$ ligands, by using AutoDock 3.0. As a result, 21 compounds were selected including known P2Y1 agonists and antagonists (Section II).

I have prepared recombinant CHO cells expressing hP2Y1 (or hP2Y2) receptors. The cells were clearly shown to increase in intracellular Ca^{2+} concentration ($[Ca^{2+}]i$) by ADP at a concentration of 10^{-6}〜$^{-9}$M. The ADP-induced Ca^{2+} response was blocked by the tentative P2Y1 antagonist, 3'P5'P adenosine (Section III). Using this assay, I have evaluated the 21 compounds mentioned above, and identified 3 noble compounds that increased the $[Ca^{2+}]i$ through activating the $hP2Y_1$ receptor stably expressed in recombinant CHO cells. Among them, 5-phosphoribosyl 1-pyrophosphate (PRPP) activated the $hP2Y_1$ receptor with the lowest ED_{50} value of 15nM. PRPP was shown to specifically activate hP2Y1 receptor.

This is the first report that demonstrates the surrogate ligands for the $hP2Y_1$ receptor were identified by using *in silico* and *in vitro* studies. Accumulation of these data can make it possible to refine the 3D structure of P2Y receptor as well as to develop a computational method to elucidate the mechanism by which a ligand binds to a GPCR. In addition, the usage of these approaches described here should be expected to discover orphan GPCR ligands.