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Development of screening systems for modulators on phospholipase-mediated signal transduction

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Many agonists have been known to activate the hydrolysis of membrane phospholipids through the bindings with corresponding receptors on the various cells. Diacylglycerol and inositol 1,4,5-trisphosphate(IP3) generated by the action of phosphoinositide-specific phospholipase C (PI-PLC) are well known second messengers for the activation of protein kinase C and the mobilization of Ca2+ in many cells. Three types of PI-PLC isozyme (β,γ, and δ) and several subtypes for each type have been identified from mammalian sources by purification of enzymes and cloning of their cDNAs. Each type PI-PLC isozyme is coupled to different receptors and mediators, for example, β-types are coupled to the seven-transmembrane-receptors via Gq family of G-proteins and γ-types directly to the receptor tyrosine kinases. Specific modulators for the signaling pathway through each type of PI-PLC should be very useful as potential candidates for lead substances in developing novel drugs.

To establish the sensitive and convenient screening systems for searching modulators on PI-PLC mediated signaling, two kinds of approaches have been tried. (1) Establishment of in vitro assay condition for each type of PI-PLC isozyme: Overexpression by using vaccinia virus and purification of each isozyme was carried out for the preparation of large amounts of enzymes. Optimum and sensitive assay condition for the measurements of PI-PLC activities were established. (2) Development of the cell lines in which each type of PI-PLC is permanently overexpressed: A fibroblast cell line (3T3γ1-7) in which PI-PLC-γ1 was overexpressed by using pZip-neo expression vector was developed and used for the measurement of PDGF-induced IP3 formation. The responses for IP3 formed in 3T3γ1-7 cells by the treatment of PDGF is 8 times more sensitive than those in control cells. 3T3γ1-7 cell is useful for the screening of the inhibitors on the PDGF-induced cellular responses from large number of samples in a small volume(50 μl) and short time(5-15 min). Using these systems, we screened hundreds of herb-extracts for the inhibition of PDGF-induced IP3 formation and selected several extracts that showed the inhibition as the candidates for isolation and characterization of active substances. The determination of the acting point of selected extracts or fractions in the PDGF signaling pathway has been analyzing.