The founding member of the centaurin protein family, rat centaurin α, was identified by its ability to bind to inositol 1,4,5-P₃ (Hammonds-Odie et al. 1996). Subsequently its higher affinity for PtdIns 3,4,5-P₃ identified it as a putative receptor for this lipid second messenger. The mammalian centaurin family has now expanded to include the related β and γ centaurin proteins. Very little is known about the function of these proteins and we are therefore exploiting the power of C. elegans as a model system to understand their functions in intracellular signalling pathways and biological processes. Two centaurin genes from C. elegans were cloned, one β centaurin, cnt-1, (chromosome II) and one γ centaurin, cnt-2, (chromosome III). The predicted C. elegans centaurins are about 33% identical to their human homologues. The protein domain structure reveals the characteristic features of centaurins: a PH domain, an ArfGAP region and ankyrin repeats. The PH domains of CNT-1 and CNT-2 have been over-expressed in E. coli and used in dot blot binding assays to determine the phospholipid binding specificity of these proteins. Both have complex phosphoinositide binding patterns. When expressed in cell lines CNT-1-PH domain:GFP fusions translocate to the membrane in response to signals that are known to induce PtdIns 3,4,5-P₃ production. The expression pattern of the C. elegans centaurin β gene is being determined using GFP reporter gene fusions. In order to investigate the function of the gene we are using RNAi.


1272 The Possible Role of p130, a New Ins(1,4,5)P₃ Binding Protein, Related to GABA Receptor Signaling in Hippocampal Neurons

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We isolated a 130-kD novel inositol 1,4,5-trisphosphate (Ins(1,4,5)P₃) binding protein (p130), similar to β-isozyme of phospholipase C, without catalytic activity. In the present study, we isolated p130 associated proteins by screening the human cDNA library using a yeast two-hybrid system. One of isolated clones was GABARAP, a GABA receptor associated protein. In order to determine the region involved in the binding between p130 and GABARAP, β-gal assay was done in yeast two-hybrid system using several deletion constructs for p130 or GABARAP. The results clearly indicate that the 221-298 residues of p130, and the 40-68 residues of GABARAP, were the sites responsible for the binding. To examine the interaction between p130 and GABARAP in vitro, pull-down assay was done using recombinant proteins of full-length p130 and GABARAP. p130 bound to a guanethidine-transfase fused GABARAP, and the binding of GABARAP to 343-404 residues of y2-subunit of GABA receptor was inhibited by p130 in a dose dependent manner, indicating that the binding of GABARAP to y2-subunit of GABA receptor would be competitive with p130. To elucidate the function of p130 on GABA receptor signalling, the effect of zinc ion on GABA receptor was investigated using whole cell patch recording using acutely dissociated hippocampal CA1 neurons from wild type and p130 knockout mice. The inhibitory action of zinc ion on GABA receptor decreased significantly in p130 mice. These results indicate that p130 could modulate the GABA receptor signalling in hippocampal neurons by changing the involvement of y2-subunit in the formation of hetero-pentameric GABA receptor.

1273 Expression of Jak/Stat signaling system in a HL60 variant cell resistant to C₂-ceramide (HL60CR) is affected by perturbation in the membrane.

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Cytokines play an essential role in the regulation of many cellular functions including cell proliferation, differentiation, and survival. Most cytokines activate Jak/signal transducer and activator of transcription (Stat) signaling pathways, which are indispensable in the regulation of various cellular programs. An initial evoking for the pathways has been thought to exclusively require a ligand-receptor interaction at the surface of the membrane. Recently, however, such membrane permeable substance as sphingolipids and those derivatives whose targets in the cell surface are not yet determined have been known to affect various signaling pathways directly, suggesting an unknown presence of mechanism controlling a signal transduction by these molecules.

Previously from human leukemia HL60 cells we established a variant cell line (HL60CR) resistant to high concentration of N-acetylphosphonoglycine (C₂-ceramide), by which maternal cells undergo prompt apoptosis. In this cell, we studied the expression of Stat1,2,3,4,5, and related molecules in the whole cell lysate, cytoplasm, and nucleus, respectively. We found that not Stat proteins but Jak1, Tyk2, Sgk-F367, and IRF2 are strongly expressed in the cells without any cytokine stimulus, while all of these proteins were scarcely expressed in the maternal cells. These results may show that CR cells are keeping high physiological activity to survive against stress leading the cells to over-express various signaling systems including Jak/Stat pathways, and furthermore raise a question as to what is essentially responsible for a continuous recruitment of signaling systems without a ligand-receptor interaction.

We considered that it might be possible if cytokine receptors and those related molecules cluster a functional domain in the membrane and the activity of these molecules are regulated by a perturbation around the cluster or itself, a drastic replacement of the component in the membrane by sphingolipids can modify the function of the cluster. Interestingly, dimethylsphingosine (DSP) caused rapid translocation of Stat3 in CR cells, contrarily addition of o-sphatidylserine suppressed the effect of DSP. This may show that perturbation of micro domain in the membrane caused by these reagents, resulted in the up- or down-regulation of Stat signaling system. Taken together, these results may support an idea that perturbation of the membrane affects Jak/Stat signaling in the cells.