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Developmental Regulation of Neuronal Phenotypes By Neurotrophic Factors And Cytokines

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A variety of intercellular signals are suggested to regulate neuronal development and contribute to synaptic plasticity. Such signals now include small chemicals , gas molecules and protein factors. In response to certain neural stimuli, these molecules are liberated from presynaptic and postsynaptic neurons, glial cells and immune cells, and synagestically affect phenotypic development of neurons and synaptic plasticity in short and long term periods. The short term actions have been often exemplified by their presynaptic influences of the signals leading to long term potentiation. Now, it is known that various protein factors including growth factors cytokines and neurotrophic factors often produce not only such short term synaptic effects but also long term affects on pre- and postsynaptic neurons by changing neurotransmitter production and their receptor expression or synaptic organization. Interestingly, some growth factors and cytokines are able to counteract with such positive factors and block their activities. Such positive and negative intercellular protein factors regulating neuronal and synaptic development are focused and their contribution to neurological and psychiatric diseases are discussed.

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Molecular Machinery of Phagocytosis and Macropinocytosis: Analysis by Fluorescence Ratio Imaging

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Both phagocytosis and macropinocytosis are actin-dependent cell motility to endocytose extracellular particles and fluid, respectively. We have been studying on the molecular machinery of phagocytosis and macropinocytosis from morphological and cytochemical aspects[1-2]. Fluorescence ratio imaging has been used to measure intracellular Ca^{2+} concentration and pH. In our studies, we applied the ratio imaging technique to analyze functional relationship between F-actin and actinin-4, a novel isoform of human α-actinin, which is associated with cell motility and cancer invasion. In mouse macrophages, actinin-4 and F-actin were doubly stained with immunofluorescence and rhodamine-phalloidin, respectively, and examined by confocal microscopy and digital image analysis. Conventional fluorescence images just showed that actinin-4 was mostly colocalized with F-actin. However, ratio images of actinin-4/F-actin further indicated that actinin-4 concentrations relative to F-actin were higher in circular ruffles, which are precursor forms of macropinosomes, than in straight linear ruffles. The actinin-4/F-actin ratio is also high around newly formed macropinosomes which could be labeled by fluid-phase pinocytosis of fluorescein-dextran. Actinin-4 was gradually dissociated from late macropinosomes during the macropinosome maturation. Similar redistribution of actinin-4 was also observed in macrophages during phagocytosis; suggesting that actinin-4 may play the same role in the two mechanistically analogous types of endocytosis. Actinin-1, a classical isoform of α-actinin also localized in F-actin-enriched regions including focal adhesion plaques, peripheral ruffles, circular ruffles and phagocytic cups. However, unlike actinin-4, the ratio of actinin-1/F-actin appeared to be more uniform. Thus, the ratio imaging indicated that actinin-4 and actinin-1 contributed differently to F-actin dynamics in macrophages, and that actinin-4 is preferentially involved in circular ruffle formation, early macropinosome maintenance and similarly in phagocytosis.

In another study, we observed contractile activity of phagocytic cups to close into intracellular phagosomes by ratio imaging of rhodamine-labeled actin/cytoplasmic volume. Macrophages were labeled by scrape-loading with fluorescein dextran and rhodamine-labeled actin. Since fluorescein dextran diffusely distributed throughout cells, the intensity of fluorescein represents cytoplasmic volume. Therefore, rhodamine/fluorescein ratio implies actin concentrations. Time-lapse video microscopic analysis of rhodamine-actin /fluorescein dextran in living macrophages showed that actin was concentrated at the distal margins of closing phagosomes. Immunofluorescence microscopy showed the presence of myosins Ic, II, V and IXb in phagosomes. Of these, only myosin Ic was concentrated at the distal margins of closing phagosomes, suggesting that myosin Ic mediates the purse-string-like contraction that closes phagosomes[2].

These studies were carried out in collaboration with Drs. Tesshi Yamada and Setsuo Hirohashi, National Cancer Center Research Institute, and Dr. Joel A. Swanson, University of Michigan Medical School.

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