ABSTRACT

Intracellular trafficking of an anti-Amyloid Protein Precursor antibody

Alzheimer's disease is characterized by over-accumulation of beta-amyloid peptide (Aβ) in the brain. Aβ is produced by proteolytic cleavage of beta-amyloid precursor protein (APP) by β- and γ-secretases. Novel monoclonal antibody, 2B12, has been shown to bind to β-secretase cleavage site of APP, reducing the production of APP, presumably by preventing the cleavage by steric hindrance. 2B12 is hypothesized to bind to APP molecules exposed on the cell surface and to be internalized in the form of complex with APP via natural endocytic pathway. This hypothesis was confirmed by San Pei Ho's (2007), who followed the internalization of 2B12 in living astrocytoma MOG-G-UVW, cells in time-course experiment.

This project is focused on intracellular trafficking of 2B12 and its localization within specific cellular compartments. Experiments were performed with fixed astrocytoma MOG-G-UVW cells (constitutively expressing APP). Originally planned experiments with live cells could not be performed due to decreased stability of 2B12 (causes remain unknown). 2B12 was tested for colocalization with polyclonal affinity purified antibodies labelling subcellular markers (proteins) associated with compartments known to participate in APP trafficking (endoplasmic reticulum, trans-Golgi network, early and late endosomes, and lysosomes). Primary antibodies were visualized by correspondent biotinylated secondary antibodies and fluorophores conjugated to avidin. Pictures were taken by fluorescent and confocal laser scanning microscope.

Based on up-to-date information of APP trafficking, 2B12 was expected to be present in all compartments mentioned above. The results proved the localization in endoplasmic reticulum, lysosomes and late endosomes. Data for early endosomes were unclear. 2B12 localization in trans-Golgi network was not confirmed.

Most of the results are in agreement with presumed 2B12 localization based on localization of APP and therefore provide another piece of evidence supporting the hypothesis of 2B12 being trafficked together with APP. Results for early endosomes and trans-Golgi network have to be revised.