Immunofluorescence Labeling of a Mutant of Tissue Non-Specific Alkaline Phosphatase Lacking the Glycosylphosphatidylinositol Anchor

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The immunofluorescence labeling of a protein in cultured cells typically involves fixation and permeabilization of the cells prior to immunoreaction. However, permeabilization may not be required for the immunolabeling of cell surface proteins. Tissue non-specific alkaline phosphatase (TNSALP) is located on the cell surface and anchors to the plasma membrane through a glycosylphosphatidylinositol (GPI) structure at the C-terminus of the protein. The cell immunofluorescence staining properties of TNSALP were investigated by preparing U2OS cells transfected with the pcDNA3-TNSALP expression vector using Lipofectamine 2000 (Thermo Fisher Scientific, Waltham, MA, USA). The cells were first fixed with formaldehyde, treated with phosphate buffered saline with or without 0.1% saponin, and then stained with TNSALP antibody. Fluorescence of TNSALP was detected on the surface of the cells. Furthermore, a strong fluorescence signal was evident at the juxtanuclear position of the saponin-permeabilized cells, showing that TNSALP protein passes through the endoplasmic reticulum and Golgi apparatus during synthesis.

A unique frame-shift mutant resulting from a T deletion at complementary DNA number 1559 (TNSALP 1559delT mutant) has an extension 80 amino acids long at its C-terminus and lacks the GPI anchor. Immunofluorescence observation of the 1559delT mutant showed the absence of fluorescence on the cell surface but the presence of fluorescence in and around the nuclei of permeabilized cells. This result indicated that the 1559delT mutant was not retained on the plasma membrane owing to a lack of the GPI anchor, although the mutant protein was processed through the endoplasmic reticulum and Golgi apparatus in a similar way to the wild-type TNSALP. This result also suggests that comparison of the permeabilized immunolabeling images with nonpermeabilized images is effective for analyzing the localization of cell-surface proteins.

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Analysis of TNSALP Lacking GPI Anchor

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Fig. 1. Immunofluorescence detection of expressed tissue non-specific alkaline phosphatase (TNSALP). The cells were cultured on a cover glass and then transfected with the TNSALP expression vector. The cells were fixed with 4% formaldehyde and then rinsed in phosphate buffered saline buffer (A) or in phosphate buffered saline buffer containing 0.1% saponin (B). The TNSALP was stained by reacting with a rabbit anti-TNSALP primary antibody (HPA008765, Sigma-Aldrich, St. Louis, MO, USA) and then labeled with a secondary antibody, Alexa Fluor® 546 conjugated goat anti-rabbit IgG (Thermo Fisher Scientific, Waltham MA, USA). The cells were counterstained with 4',6-diamidino-2-phenylindole using ProLong® Gold antifade reagent (Thermo Fisher Scientific).

Fig. 2. Immunofluorescence detection of the tissue non-specific alkaline phosphatase (TNSALP) mutant. Immunostaining was performed as described in Figure 1. Cells were transfected with the following expression vectors: WT, wild-type TNSALP; 1559delT, mutant TNSALP lacking the glycosylphosphatidylinositol anchor; mock, only pcDNA3 vector (negative control).

References