—Photogravure—

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

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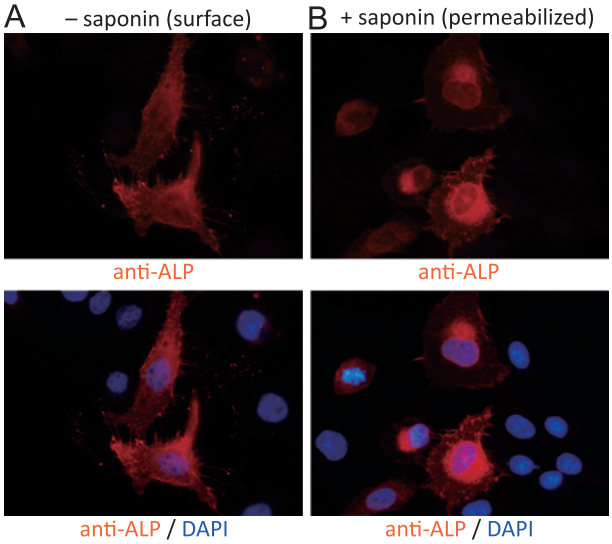

Fig. 1

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[1]. 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 (**Fig. 1**). Fluorescence of TNSALP was detected on the surface of the cells (**Fig. 1A and B**). Furthermore, a strong fluorescence signal was evident at the juxtanuclear position of the saponin-permeabilized cells (**Fig. 1B**), showing that TNSALP protein passes through the endoplasmic reticulum and Golgi apparatus during synthesis[1,2].

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[2,3]. 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 (**Fig. 2**). 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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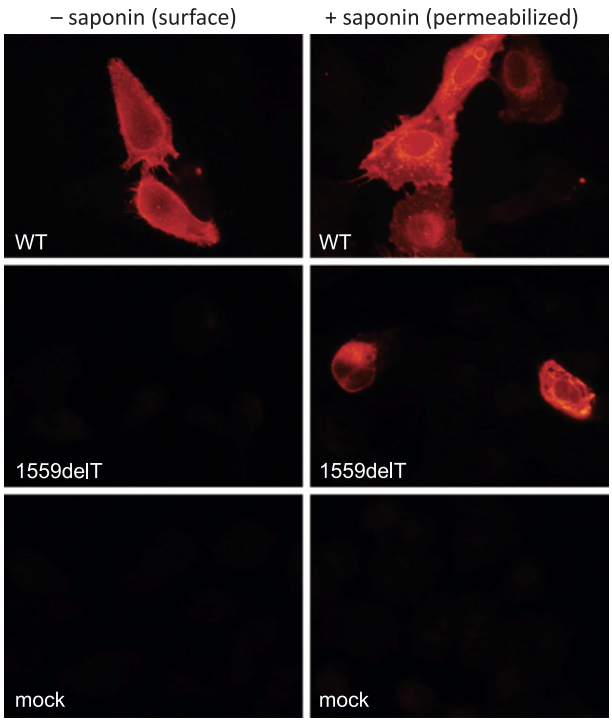

Fig. 2

Conflict of Interest: The authors declare no conflict of interest.

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

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