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PO-008 - (23883) - DYNAMICS OF DORSAL CELL SURFACE OF CULTIVATED CELLS STUDIED BY SCANNING ION CONDUCTANCE MICROSCOPY

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Culture cells often extend lamellipodia and filopodia at the cell edges, but also acquire cytoplasmic protrusions (villous protrusions, ruffles etc.) on their apical surface. These structures have been observed by scanning electron microscopy (SEM); however, their dynamics remain unclear. This study introduced a time-lapse imaging technique using scanning ion conductance microscopy (SICM) to study the apical cell surface dynamics of live cultured cells. Simultaneously, fluorescence microscopy was applied to analyze cell activity in relation to actin dynamics beneath the cell membrane.
Cells from human gingival carcinoma cell line (Ca9-22) were transfected with linearized plasmids for EGFP-actin. Using SICM combined with an inverted optical microscope, we obtained both topographical SICM images and fluorescent images of the actin-GFP expressing cultivated cells.
SICM revealed the three-dimensional ultrastructure of apical surface ruffles in the live cells, while the intracellular actin filaments were simultaneously visualized by fluorescence microscopy. The time-lapse SICM images clearly showed the constant movement of the ruffles under suitable conditions. Fluorescent images also showed the movement of these structures in relation to the dynamics of the intracellular actin filaments; the ruffles shortened or disappeared following treatment with the actin polymerization inhibitor latrunculin-A.
Combination of SICM and fluorescence microscopy is useful for analyzing the movement of the apical cell surface in relation to the actin filament dynamics. Our findings also indicate that accumulation of actin filaments beneath the cell membrane is related to the production and movement of the apical cell protrusions.

Palavras-chave : Scanning Ion Conductance Microscopy,, Apical Cell Surface,, Actin Filament Dynamics