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Different methods of detaching adherent cells significantly affect the detection of TRAIL receptors

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ABSTRACT

Aims and background. As a powerful technique allowing analysis of large numbers of cells, fluorescence-activated cell sorting (FACS) is used more and more widely. For FACS analysis, adherent cells are usually detached by trypsinization, followed by centrifugation and resuspension. However, trypsinization can cut off some receptors from the cell surface like fine scissors, which will affect the accuracy of FACS results. Though non-enzymatic methods such as citric saline buffer have been used to determine cell surface receptors, how much of the receptors is cut off by trypsinization has been rarely studied. This work aimed to investigate whether different methods of detaching adherent cells could affect the detection of cell surface receptors.

Methods. Human hepatocellular carcinoma cell lines (HepG2, Huh7 and Hep3B) were detached enzymatically with trypsin-EDTA solution or non-enzymatically with citric saline buffer, and then the receptors of tumor necrosis factor-related apoptosis-inducing ligand (TRAIL) were detected by FACS analysis. Cell viability, cell cycle and apoptosis (sub-G1 fraction detected by FACS) of the trypsin-EDTA group and citric saline buffer group were also studied.

Results. Different methods of detaching adherent cells could significantly affect the detection of TRAIL receptors. Compared to the conventional trypsin-EDTA group, the non-enzymatic group showed a 3.42-fold increase in the mean fluorescence intensity index of DcR HepG2 and a 1.25-fold increase in DR Huh 7 (P <0.05). However, the viability, cell cycle and apoptosis of these cells were not affected.

Conclusions. Citric saline buffer might be recommended as the first choice to detach adherent cells for FACS analysis of cell surface receptors.

Key words: citric saline buffer, death receptor, fluorescence-activated cell sorting (FACS), tumor necrosis factor-related apoptosis-inducing ligand (TRAIL).

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