Evaluation of the transfection efficacy of plasmid DNA by fluorescence-activated cell sorting and confocal microscopy

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Introduction

In order to study protein-protein interactions in mammalian cells we used BiFC (Bimolecular Fluorescence Complementation) method, a key technique to visualize protein-protein interactions in model organisms. Based on the reconstitution of a fluorescent protein *in vivo*, we confirmed the interaction of two proteins in mouse embryo fibroblast cells (NIH 3T3); SH2 domain-containing protein 3C (SH2D3C) and dipeptidyl peptidase 3 (DPP3). SH2D3C protein acts as an adapter protein involved in the regulation of cell adhesion and migration, tissue organization, and the regulation of the immune response. DPP3 is a Zn metallopeptidase known to cleave dipeptides from N-termini of 3-10 aa long peptides. Through the known interaction with KEAP1, DPP3 is involved in the regulation of oxidative stress response through Nrf2/KEAP1 signaling pathway. The preliminary co-localization analysis of the EGFP-DPP3 and SH2D3C-mCherry in NIH 3T3 cells showed that the 2 chimeric proteins co-localize in cytoplasm and on the membrane (in membrane ruffles) which indicates the possible involvment in the control of cell migration. NIH 3T3 cells were transfected with BiFC plasmids but we confronted the issue of low transfection efficacy with several transfection reagents (Lipo2000, PEI, LTX, Xfect). Therefore, we attempted to evaluate delivery efficiencies by EVOS Floid, fluorescence microscopy, confocal microscopy and flow cytometry, and have faced distinct problems with each of the method.

Co-localization analysis

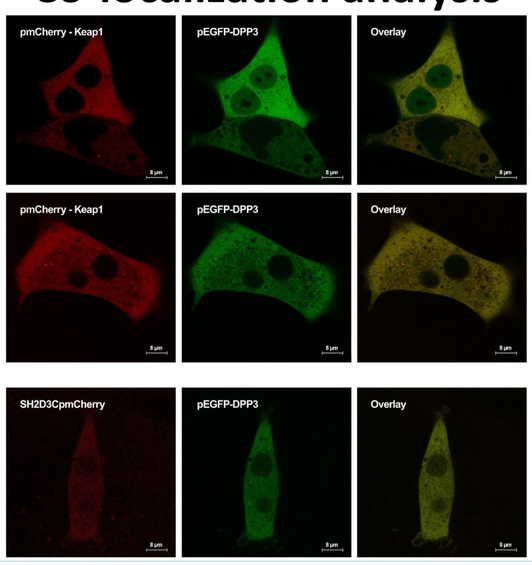

Fig. 1. Co-localization of mCherry-Keap1 with EGFP-DPP3, and SH2D3C-mCherry with EGFP-DPP3 in NIH 3T3 cells. Cells were seeded in 4 chamber glass bottom dish (IBL) and analyzed on EVOS Floid. The suitable cell confluency was 30–40% at the time of transfection. The ratio of DNA (µg) to LTX (µL) was 1:2.

BiFC analysis of DPP3-VenusfN and SH2D3C-VenusfC

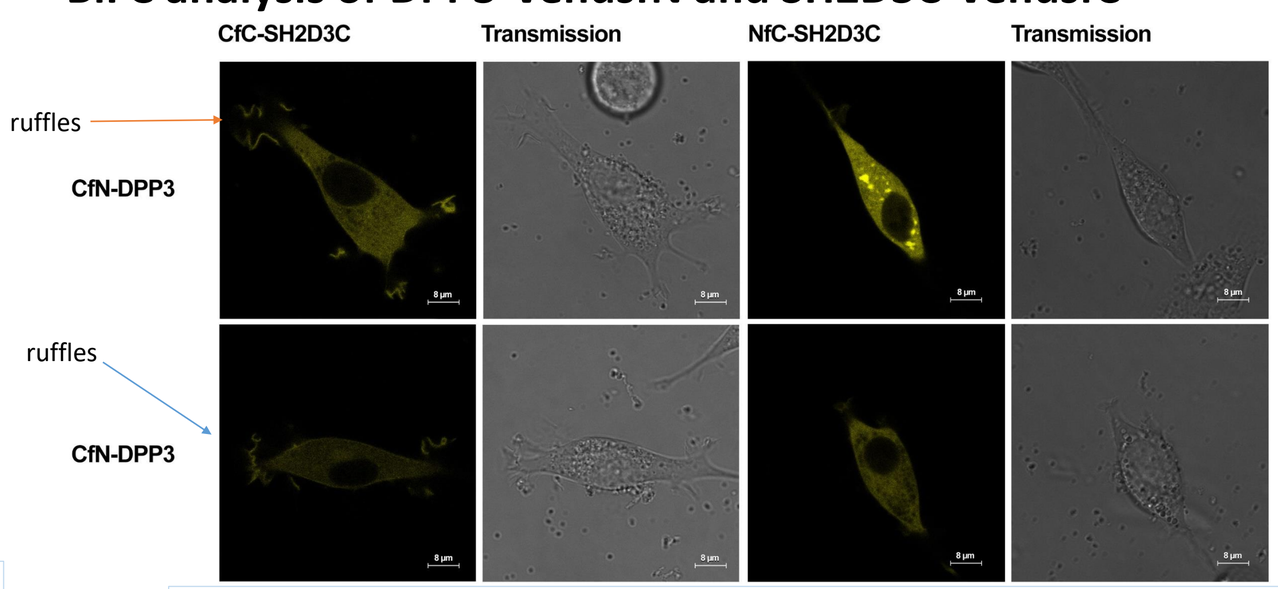

Fig. 2. NIH 3T3 cells were transiently transfected with vectors expressing DPP3-VenusfN and SH2D3C-VenusfC. Live cells were analyzed by TCS SP8 X confocal microscopy. BiFC results show that the interaction of DPP3 and SH2D3C localizes in cytosol, but also in the membrane ruffles which may be the indication of its involvement in the control of cell migration. The glass dishes, cell confluency and ratio of DNA (µg) to LTX (µL) were shown on Fig. 1.

Analysis of transfection efficacy by flow cytometry in NIH 3T3 cells

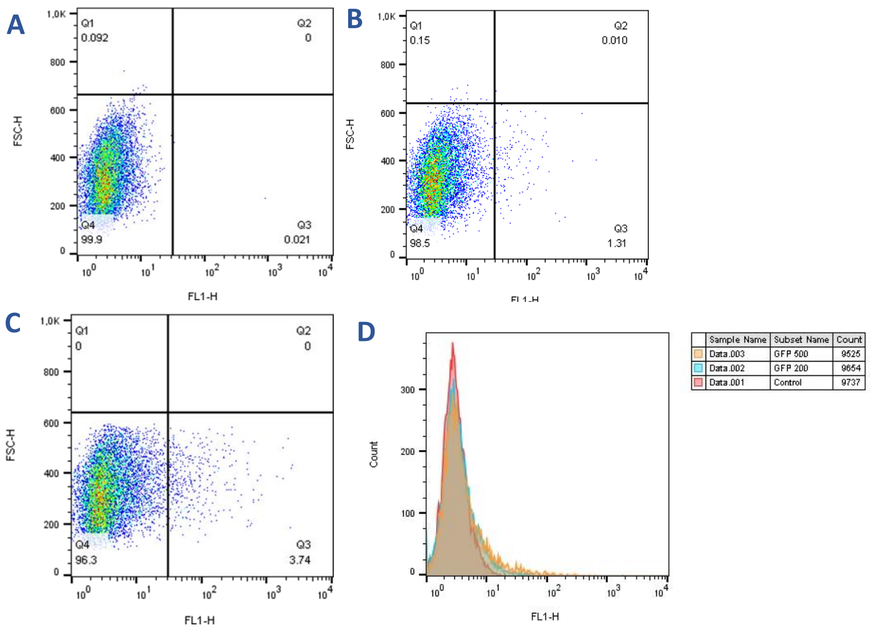

Fig. 3. FACS dot plots and histogram. (A) non-transfected NIH 3T3 cells (control group). Cells were transfected with 250 ng EGFP-DPP3 (B) and 500 ng EGFP-DPP3 (C) with Xfect reagent according to standard procedure. The overall transfection efficacy was quantitatively detected as percentage of fluorescent cells (Quadrant 3) using flow cytometry (BD FACSCalibur; Becton Dickinson, Franklin Lakes, NJ, USA). The percentage of EGFP-expressing cells was analyzed in 1×10^4 cells. Data was analyzed by CellQuest software (Becton Dickinson). The transfection efficacy of EGFP-DPP3 expressing cells (B) was 1.31% and (C) 3.74%, respectively. Time: 48 h after transfection. (D) histogram showing no change in expression between control group cells and cells expressing EGFP-DPP3 protein.

Results:

In summary, based on the FACS results, NIH 3T3 cells are not the best approach for BiFC analysis because of the transfection procedure that is relatively poor. However cells are easily distinguishable by confocal microscopy. Contrarily, HeLa cells showed high transfection efficacy and are therfore a good starting point for visualizing protein-protein interactions by BiFC that we are planning to perform.

References:

Prajapati, S.C. et al. *FEBS J*. (2011) 278(18): 3256–3276.
Mace, P.D. et al. *Nat. Struct. Mol. Biol*. (2011) 18(12): 1381–1387.

Analysis of transfection efficacy by flow cytometry in HeLa cells

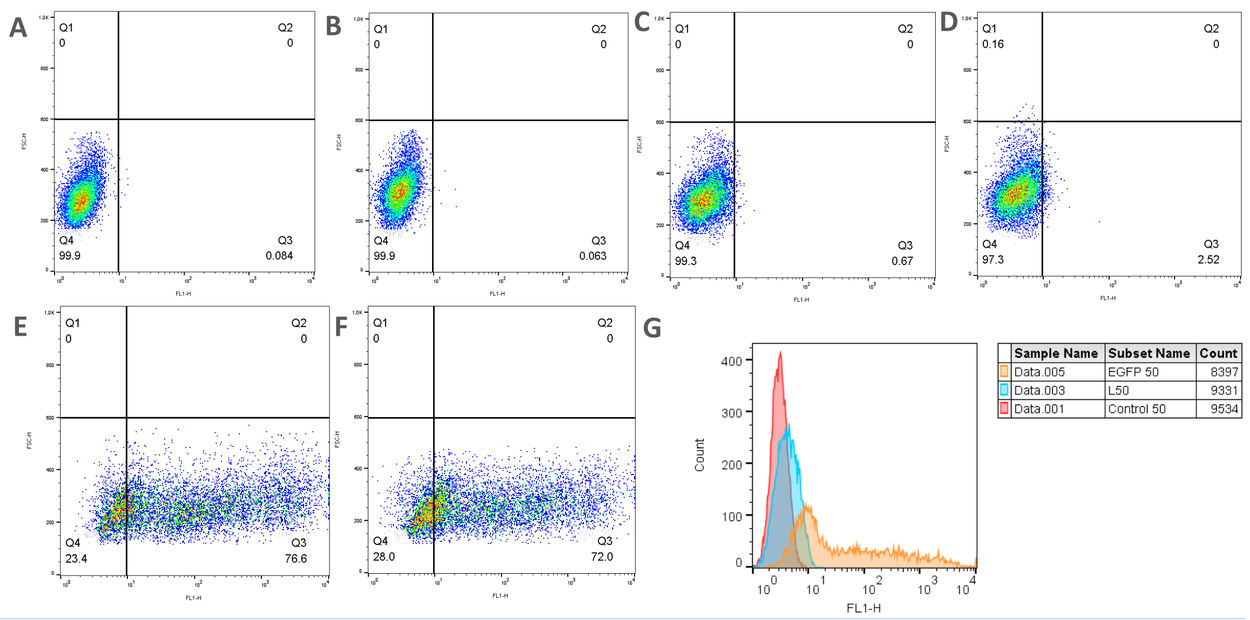

Fig. 4. FACS plots and histogram. Cells were seeded in different concentrations in 24-well plates; 25.000 (A, C, E) and 50.000 (B, D, F) cells per well. (A,B) non-transfected HeLa cells (control group). (C) and (D) histograms relate to cells treated only with transfection reagent (as an additional control), while (E) and (F) histograms show cells transfected with 500 ng EGFP-DPP3. Cellular transfection efficiencies mediated by Lipofectamine™ 2000 appeared to be significantly higher then those calculated in Fig. 3. and were about 76,6% (E) and 72% (F), respectively, but in HeLa cells.

Comparison of transfected efficacy of HeLa *vs* NIH 3T3 cells

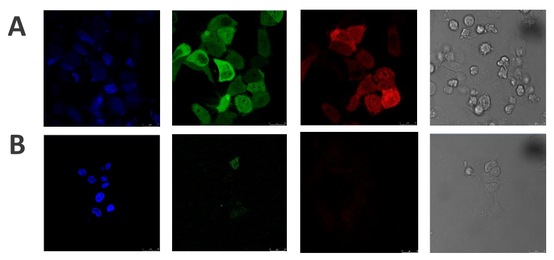

Fig. 5. Transfection efficacy. Confocal photofluoro-micrographs of (A) HeLa and (B) NIH 3T3 cells stained with Hoechst 33342 (blue); and transfected with pEGFP-DPP3 (green) and pmCherry-SH2D3C (red) plasmids. For each transfection with (A) Lipofectamine 2000 or (B) Xfect, (A) 20,000 cells/well and (B) 8,500 cells/well were distrubuted in glass bottom petri dish and incubated 24h in 5% CO_2 at 37°C. Tranfected cells were also incubated 24h. prior to confocal microscopy Hoechst dye 33342 was incubated 10 min.