Plasmid DNA-transfection of human fibrosarcoma cells FLYRD18 using Biontex-K2®Transfection System

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Plasmids

pSFG and pSERS11 (modified to be self-inactivating) are both gammaretroviral vectors derived from the Moloney murine leukemia virus and carry the same chimeric antigen receptor (CAR).

Cell culture

The human fibrosarcoma cell line FLYRD18 (retroviral producer cell line derived from HT1080 cells) was cultured in antibiotic-free Isecove's modified Dulbecco's medium (IMDM, Sigma-Aldrich) containing 10% fetal bovine serum. For transfection 1.8×10^6 cells/plate were seeded in a 100 mm tissue culture plates, with a culture volume of 5 ml per plate. Cells were incubated 24 h at 37°C in a CO_2-incubator. Transfection was performed at a cell confluence of 60-80%.

Biontex-K2®Transfection

All reagents (plasmid DNA, K2® Multiplier and K2®Transfection Reagent) were brought to room temperature before the experiment. 50 µl K2® Multiplier was carefully dropped onto the respective plates two hours before transfection. Mixing was achieved by gently agitating the culture plates. The plasmid used for transfection were either pSFG_CAR or pSERS11_CAR. For each plate of FLYRD18 cells 3.75 µg of the respective plasmid and 50 µl of the K2® Transfection Reagent were each mixed with 300 µl serumfree IMDM (Solution A and B) by a single up- and down pipetting step. Both solutions were then combined, carefully mixed and incubated for 15 minutes at room temperature before being added dropwise to the cells. Cells were incubated for 18 h at 37°C and 5% CO_2 and then refed with 5 ml fresh culture medium (Panserin).

Genejuice®Transfection

All reagents (plasmid DNA and Genejuice®; Merck) were brought to room temperature before the experiment. The plasmid used for transfection were as above. For each plate of FLYRD18 cells 30 µl of the Genejuice® transfection reagent was mixed with 500 µl serumfree IMDM by a single up- and down pipetting step and incubated for 5 minutes at room temperature. Then 3.75 µg of the respective plasmid was added, again mixed by a single up- and down pipetting step and incubated for 15 minutes at room temperature before being added dropwise to the cells. Cells were incubated for 18 h at 37°C and 5% CO_2 and then refed with 5 ml fresh culture medium (Panserin).

Analysis of transfection efficiency

The transfected FLYRD18 cells were detached from the plates after additional 48 h, washed once with WB (PBS 0.2% BSA), resuspended in 100 µl WB and stained with the anti-CAR-PE antibody for 15 minutes in the dark at room temperature. After a final washing step, the cells were resuspended in 300 µl 1% Formaldehyd and incubated for 30 minutes at 4°C prior to analysis by flow cytometry.

Figure 1: Transfection efficiency FLYRD18 cells were analyzed 72 hours after transfection for CAR expression by flow cytometry.

Conclusion

FLYRD18 cells are difficult to transfect, even more so with a self-inactivating retroviral vector. While we observed more or less comparable transfection efficiencies with pSFG_CAR, only the K2® Transfection Reagent was able to transfect the FLYRD18 cells with the pSERS11_CAR.