Transient transfection of GD25-β_1 mouse embryonal fibroblasts using METAFECTENE transfection reagent

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Cell line

GD25-β_1 cells are derived from the knock-out cell line GD25, which is deficient in integrin β_1 expression[1]. GD25-β_1 cells have been stably re-transfected with the integrin β_1 gene and are used as control cells.

Protocol

The medium of cells at 90-100% apparent confluency was exchanged for DMEM with 10% FCS without antibiotics. For transient transfections in a 3.5 cm culture dish, 5 µg of plasmid DNA and 10 µl metafectene were mixed in a final volume of 200 µl DMEM without FCS and antibiotics. This mixture was incubated for 15 – 20 min at room temperature, before being added to the cells. The cells were then incubated for 48 h with the DNA/lipid complexes before being subjected to flow cytometry.

For FACS analysis 8 x 10^5 cells were detached with PBS / EDTA and resuspended in 400 µl of PBS. 20 µl of FITC-conjugated anti-α_2 antibody were added and the cells were incubated in the dark on ice for 1h. The cells were washed three times with 1 ml PBS and then resuspended in 400 µl of PBS (2 x 10^6 cells per ml). FACS analysis was done using a BD FACScan analyser at a wavelength of 515 – 545 nm.

Discussion

We have established that using 5 µg of plasmid DNA and 10 µl metafectene per 3.5 cm dish results in a transfection efficiency of about 50% as determined by flow cytometry (Fig. 1a). This applies to the GFP-tagged aminoglycoside phosphotransferase, which is expressed from a SV40 promotor. The transmembrane protein integrin α_2 appears to be expressed in a percentage of cells slightly smaller than 50% (Fig. 1b). Both proteins are expressed from a plasmid derived from pCMV-Script® (Stratagene).

Next, we will attempt to establish a stable cell line with the plasmid used here for transient transfection and use METAFECTENE for screening mutants transiently expressed in these cells.

Results

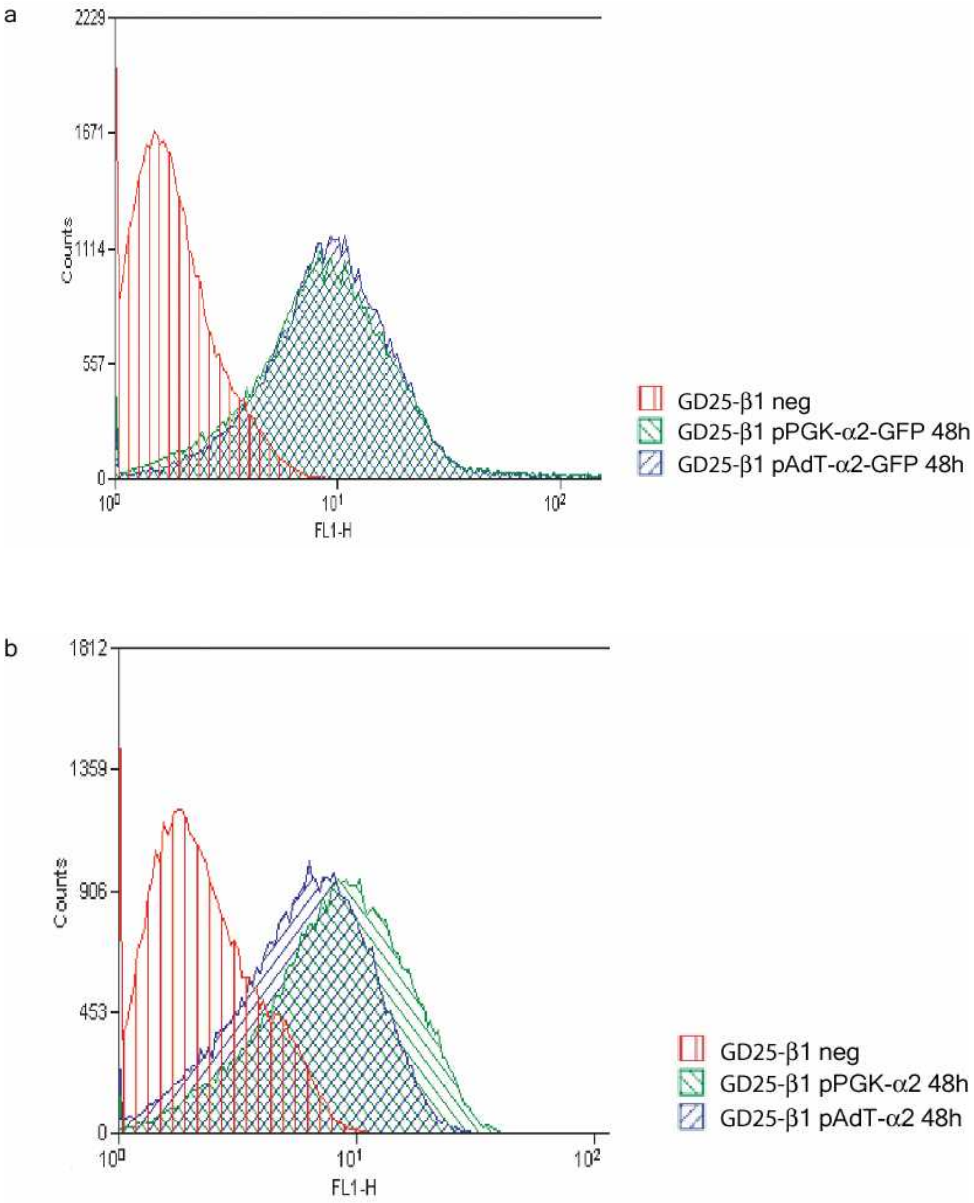

Fig. 1: Histograms of FACS analysis of transiently transfected GD25 cells 48 hours after transfection.

1. Fassler, R., *et al.* Lack of beta 1 integrin gene in embryonic stem cells affects morphology, adhesion, and migration but not integration into the inner cell mass of blastocysts. *J Cell Biol*, **128**(5): 979-88.