

Transfection of human glioma cell lines

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Introduction:

We transfected two human glioma cell lines (LNT-229, U87MG) with pcDNA3 or pcDNA3-EGFP as a control, or with pCMV-hu-NTN1 to generate stable cell lines.

Materials and methods;

Cell culture components were purchased from PAA (Pasching, Austria), Metafectene EASY and Metafectene PRO from Biontex (Munich, Germany); anti-Human Netrin-1- antibody (Enzo Life Sciences, Lörrach, Germany). Secondary antibodies were purchased from Santa Cruz.

Experimental procedures / transfection protocol:

The cells were cultured in DMEM (10% FCS, 10 µg/ml Pen/Strep) at 37°C, 5% CO₂. 60.000 cells/well were seeded in 12 well plates and allowed to attach overnight. Transfections were done according to the manufacturer's protocol using either Metafectene PRO or Metafectene EASY. 48h after transfection, the medium was changed to selection medium (10 µg/ml G418 sulfate). Transfection efficacy was evaluated at the same time point by optically using fluorescence microscopy counting EGFP-positive cells. Surviving cells were pooled. Protein concentration of cell lysates was determined by the Bio-Rad BCA assay and 20 µg of protein was loaded per lane on a SDS-PAGE. Transgene expression was assessed by immunoblot using a mouse anti-NTN-1 antibody (clone Nora1).

Results and discussion:

The transfection efficacy was approximately 60% for U87MG cells using Metafectene PRO and 80 % using Metafectene EASY. For LNT-229 cells, transfection efficacy was approximately 50% using Metafectene PRO and 70% using Metafectene EASY (data not shown). Pooled surviving cells transfected with pcDNA3-huNTN1 showed well expression of NTN1.

Conclusion / summary:

Metafectene EASY is a feasible reagent for the transfection of human glioma cell lines, Transfection efficacy using Metafectene EASY is better than using Metafectene PRO. The transfection protocol is easy to handle with.

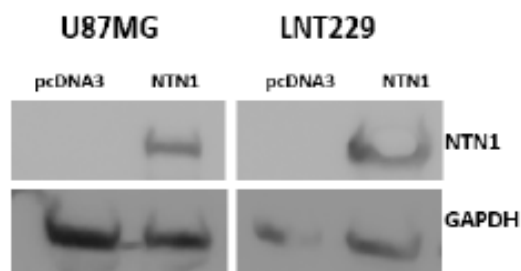


Fig. 1: Transfection of U87MG or LNT-229 cells with pcDNA3 or pCMV-NTN1 (NTN1, human Netrin-1; GAPDH, glyceraldehydephosphate-dehydrogenase)