DNA-transfection of HeLa cells using "Biontex K2® Transfection System"

Dr. med. Maximilian Linxweiler, Department of Otorhinolaryngology, Saarland University Medical Center, Kirrbergerstrasse 100, 66421 Homburg/Saar, GERMANY

Materials and Methods

Cell Culture

HeLa cells (ATTC® CLL-2™) were cultured at 37°C in DMEM-medium (Gibco Invitrogene, Karlsruhe, Germany) containing 10% FBS (Biochrom, Berlin, Germany) and 1% penicillin/streptomycin (PAA, Pasching, Austria) under humidified environment with 5% CO_2.

Plasmid Transfection

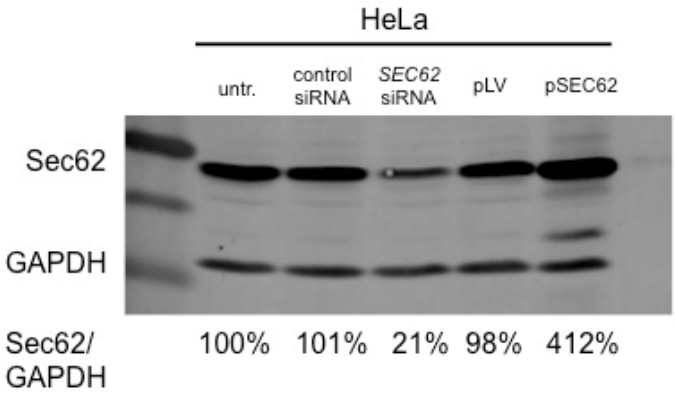
(find accurate reagent amounts in the table below)

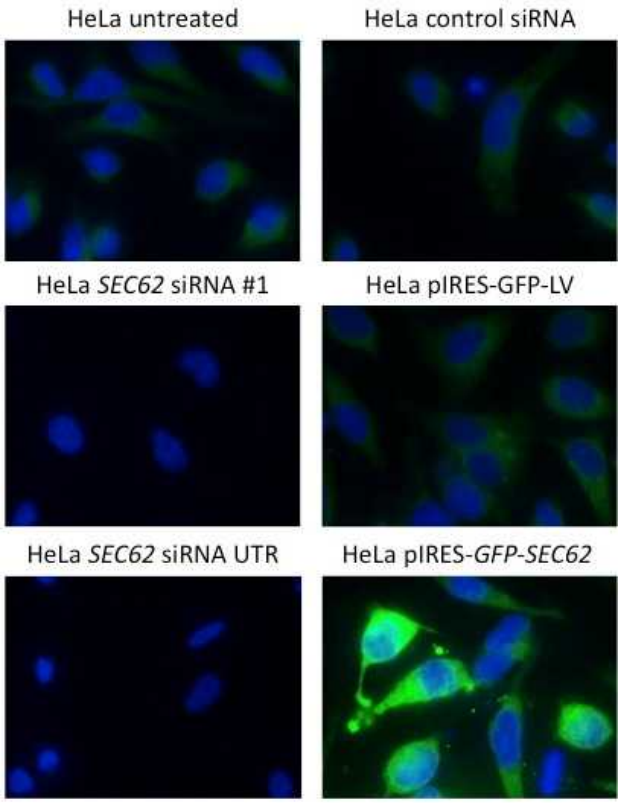
For overexpression of plasmid encoded *SEC62* gene 2.4 x 10^5 cells per well of 6-well plates were seeded in normal culture medium as described above. The cells were transfected with either the IRES-GFP-Sec62 plasmid or with the IRES-GFP-LV plasmid as negative control using the K2® transfection system.
Cells were treated with K2® Multiplier, 2h before DNA transfection. For this K2® Multiplier was dripped slowly onto the medium and mixed by gently swaying the dishes. K2® Transfection Reagent was mixed with Opti-MEM® reduced serum medium (Gibco Invitrogene, Karlsruhe, Germany) and left on room temperature during preparation of the DNA. Plasmid-DNA encoding *GFP-SEC62* (GFP-tagged Sec62 protein) was mixed with Opti-MEM®. DNA-solution was added to the solution containing the K2® Transfection Reagent and mixed by inverting the tubes, followed by 15 minutes incubation at room temperature. Transfection solution was applied to the cells by slow dropwise addition to the medium followed by gently swaying the dishes. The transfected cells were again incubated as described above for 48 to 72 hours. Overexpression of the plasmid encoded gene was evaluated by western blot analysis as well as immunofluorescence. The maximum overexpression effect was seen 72 h after the plasmid transfection.

Dish size	DMEM	K2® Multiplier	K2® Transfection Reagent	Opti-MEM for K2® Transfection Reagent/DNA	DNA (pIRES-*GFP-SEC62*)
34,8mm	1,5ml	10µl	2µl	56µl/56µl	2µg

Results

Immunofluorescence & Western Blot





Conclusions

For the Western Blot and immunofluorescence experiments HeLa cells were transfected with an IRES-*GFP-SEC62* plasmid or an IRES-*GFP*-LV plasmid using the K2® Transfection system. The blot showed a band of the expected size (ca. 46kDa). GAPDH was used as internal loading control (ca. 37 kDa). When referring the level of Sec62 to GAPDH, the plasmid transfection increased the cellular Sec62 level up to 412% as compared to cells transfected with a control plasmid (IRES-*GFP*-LV plasmid). To test the specificity of the Sec62 antibody (self-produced polyclonal rabbit peptide antibody), the cells were additionally transfected with two different *SEC62*-siRNAs (*SEC62* siRNA #1, *SEC62* siRNA UTR) leading to a reduction of Sec62 protein level to 21%. Fluorescence microscopy using a FITC-labeled anti-rabbit secondary antibody showed a successful transfection in about 70-80% of the cells. The vitality of the cells was not affected by plasmid transfection as compared to untreated control cells (vitality was tested by the Countesse® cell counter; life technologies) and always exceeded a border of 93% vital cells. Taken together, the data showed high transfection rates and a sufficient overexpression of plasmid encoded *SEC62* gene without any cytotoxic effects of the transfection system.