Transfection of human podoctes with micro-RNA mimics by using the "Biontex K2®Transfection System".

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Materials and Methods

Human podocytes (obtained from Prof. Dr. Pavenstädt, Münster, Germany) were cultured with 10 ml growth medium: RPMI 1640 medium with L-glutamine (Lonza, Belgium) supplemented with 10% fetal calf serum (pH 7.0-7.5; GIBCO™ Invitrogen Corporation, Germany) and 1% penicillin-streptomycin (GIBCO™ Invitrogen Corporation, Germany) as well as Insulin-Transferrin-Sodium selenit supplement (0.1 mg/mL; Roche, Switzerland), HEPES buffer (5 mM; GIBCO™ Invitrogen Corporation, Germany), NEAA (1X; Cambrex, USA) and sodium pyruvate (100 nM; GIBCO™ Invitrogen Corporation, Germany) in 75 cm² CELLSTAR® Cell Culture Flasks (Greiner Bio-One GmbH, Germany) at 37°C and 5% CO^2. 12 Glass plates (12 mm diameter; Carl Roth GmbH & Co. KG, Germany) were seeded in Cell culture dishes (60x15mm; Greiner Bio-One GmbH, Germany) and coated with mouse collagen type IV (BD Bioscience, Germany) for 30 minutes and subsequently washed two times with 1x PBS Dulbecco without Ca^{2+} and Mg^{2+} (Biochrom, Germany). Cells were seeded on the coated slides in 4 ml medium after trypsination. A confluence of 60-70% was obtained after 3 days at 37°C and the plates were transferred to a 24-well cell culture plate (Greiner Bio-One GmbH, Germany). 500 µl medium was added prior to transfection.

Transfection

A Cy3-labeled, scrambled micro-RNA (10 µmol) was thawed on ice and added to RPMI 1640 medium with L-glutamine without supplements (solution A). K2® Transfection reagent was added to medium without supplements accordingly (solution B). Solution A was added to solution B and both were gently mixed by pipetting up-and down once. After 20 minutes of incubation at room temperature the transfection mix was slowly given into the wells containing the glass dishes with human podocytes and medium containing supplements. After 24 h at 37°C and 5% CO^2 the medium with transfection solution was removed and 500 µL fresh medium with supplements was added. This was followed again by incubation for 24 h at 37°C and 5% CO^2. A Alexa Fluor® 546-Phalloidin and DAPI staining was performed afterwards in order to visualize the cytoskeleton and the nuclei.

	Solution A		Solution B	
Growth medium per well	RPMI 1640 Medium with L-glutamine without supplements	miRNA (60-70% confluent cells)	RPMI 1640 Medium with L-glutamine without supplements	K2® Transfection reagent
500 µl	30 µl	1,5 µl	30 µl	1.95 µl

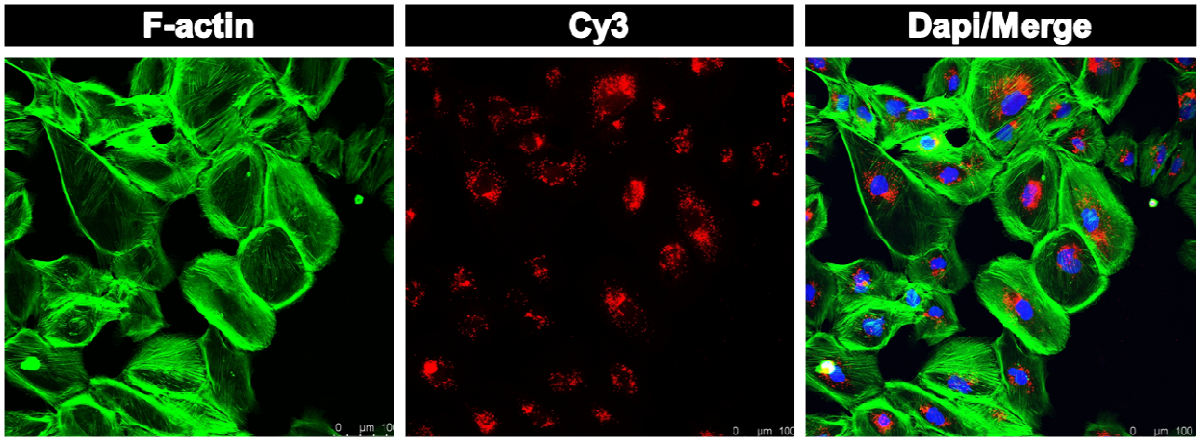

Figure 1: Fluorescence staining of human podocytes with transfected, Cy3-labeled miR. Cultured human podocytes were stained with Alexa Fluor® 546-Phalloidin (F-actin, green) and DAPI (nuclei, blue).

Conclusions

The transfection of micro-RNAs in cultured human podocytes was successfully performed with the K2®Transfection System. A transfection efficiency of nearly 100% was achieved by using the described concentrations and cell densities. Previous experiments with higher cell densities have shown similar results. Neither cytotoxic effects nor changes in cell morphology were observed in comparison to non-treated cells.