DNA transfection of renal epithelial cells, human primary cells and human cell lines, using “Biontex K2®Transfection System”.

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Materials

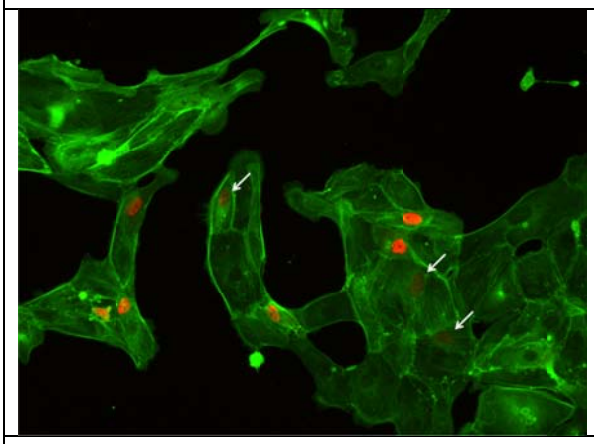
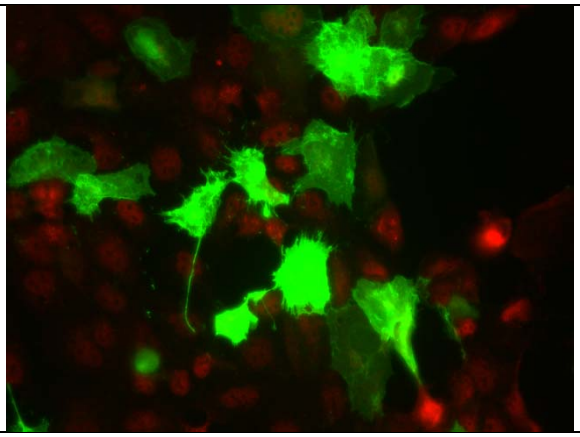
K2® Multiplier (Biontex Laboratories)
K2® Transfection reagent (Biontex Laboratories)
Opti-MEM® Reduced Serum Medium (Life Technologies)

Cell culture and transfection

Primary human tubular epithelial cells were isolated and cultured as described previously [1]. The human proximal tubular cell line HKC-8 was kindly provided by S. Racusen [2] and cultured as described [3]. Primary cells (100 000 cells) or HKC8 cells (80 000 cells) were seeded in a 24 well plate on collagen IV-coated glass cover slips in serum containing medium to allow adherence. The next day, medium was changed to 0 % serum, corresponding to the normal growth medium of epithelial cells. Concomitantly, K2® Multiplier (5 µl / 0.5 ml) was added to the wells. After 2 h, the complex of DNA and K2® transfection reagent was added, which had been prepared 20 min before: in a 96 well plate, DNA (0.5 µg) was diluted in 15 µl Opti-MEM® Reduced Serum Medium (Life Technologies); in another well, 15 µl of Opti-MEM® were mixed with 1.2 µl of K2® transfection reagent by pipetting once up and down. Subsequently, the diluted DNA was added and mixed by pipetting once up and down. After 15 – 20 min incubation at room temperature the mix was added to the cells (30 µl / 500 µl). 24 h later, cells were fixed by PFA and proteins of interest were detected by immunocytochemistry using standard techniques.

References:
1. Kroening S, Neubauer E, Wullich B, Aten J, Goppelt-Struebe M (2010) Characterization of connective tissue growth factor expression in primary cultures of human tubular epithelial cells: modulation by hypoxia. Am J Physiol Renal Physiol 298: F796-806.
2. Racusen LC, Monteil C, Sgrignoli A, Lucskay M, Marouillat S, et al. (1997) Cell lines with extended in vitro growth potential from human renal proximal tubule: characterization, response to inducers, and comparison with established cell lines. J Lab Clin Med 129: 318-329.
3. Kroening S, Neubauer E, Wessel J, Wiesener M, Goppelt-Struebe M (2009) Hypoxia interferes with connective tissue growth factor (CTGF) gene expression in human proximal tubular cell lines. Nephrol Dial Transplant 24: 3319-3325.

Results

	
Primary tubular epithelial cells were transfected with the transcriptional activator MKL (nuclear localization, red fluorescence); F-actin fibers were visualized with rhodamine-phalloidin (green). Arrows indicate cells with lower expression levels of the transgene.	HKC-8 cells were transfected with dominant negative Rac1 (flag tag detected in green). Endogenous MKL was detected by specific antibodies (pseudo color: red).

Over time, we had tested several transfection reagents most of which were not suitable for transfection of primary cells with transfections efficiencies of 10 – 20 cells per plate. Also with K2® transfection reagent transfection efficiencies of primary epithelial cells were not comparable with easy to transfect cell lines, but reached 5-10 %. Viability of the cells was barely affected after 24 h, whereas cells looked less healthy after 48 h. Therefore, we restricted our experiments to 24 h whenever possible.

HKC-8 cells are less difficult to transfect, but also in the lower range of about 10 %. The cells tolerated the transfection very well and were used after 24 without any signs of loss of viability or stress. Transfected HKC-8 cells were also used in reporter gene assays. Transfection efficiency was very reliable based on the enzyme activity of the control plasmid beta-galactosidase: 0.504 $\pm$ 0.007 OD, means $\pm$ SD of 7 wells transfected in parallel.