

Angiotensin Converting Enzyme 2 Silencing Alters Podocyte Cytoskeleton

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INTRODUCTION AND AIMS

Renin angiotensin system (RAS) blockade has been shown to be effective in delaying diabetic nephropathy progression⁽¹⁾. Podocytes are key cells in glomerular filtration barrier with their own RAS. Our group previously published the direct protective effect of insulin on diabetic podocyte by differently modulation of RAS, fibrosis and apoptosis⁽²⁾. In the puromycin model of podocyte damage, an imbalance toward a more dynamic actin cytoskeleton and increased migration could underlie podocyte dysfunction⁽³⁾.

Our objective is to study the effect of Angiotensin Converting Enzyme 2 (**ACE2**) or Insulin Receptor (**InsR**) gene silencings in the growing and migration capability of podocytes. Furthermore the expression of genes related to fibrosis was also tested.

METHODS

A conditionally immortalized mouse podocyte cell line was used. Cell proliferation was allowed by permissive conditions with mouse γ -interferon at 32°C for 3-5 days. After that, cells were induced to differentiate for 10 days, in non-permissive conditions, at 37°C and removing γ -interferon from the medium.

Cells were then transfected with ACE2-specific siRNA or InsR-specific siRNA (Silencer® Select, Thermo Scientific). After 24 hours, live-time series were continuously recorded using Zeiss Cell Observer HS microscope within an incubation chamber (37°C, 5%CO₂) for 17h. Comparisons were done between 0h and 17h of recording. Cells were stained for F-actin. Image processing was done by ImageJ.

RESULTS

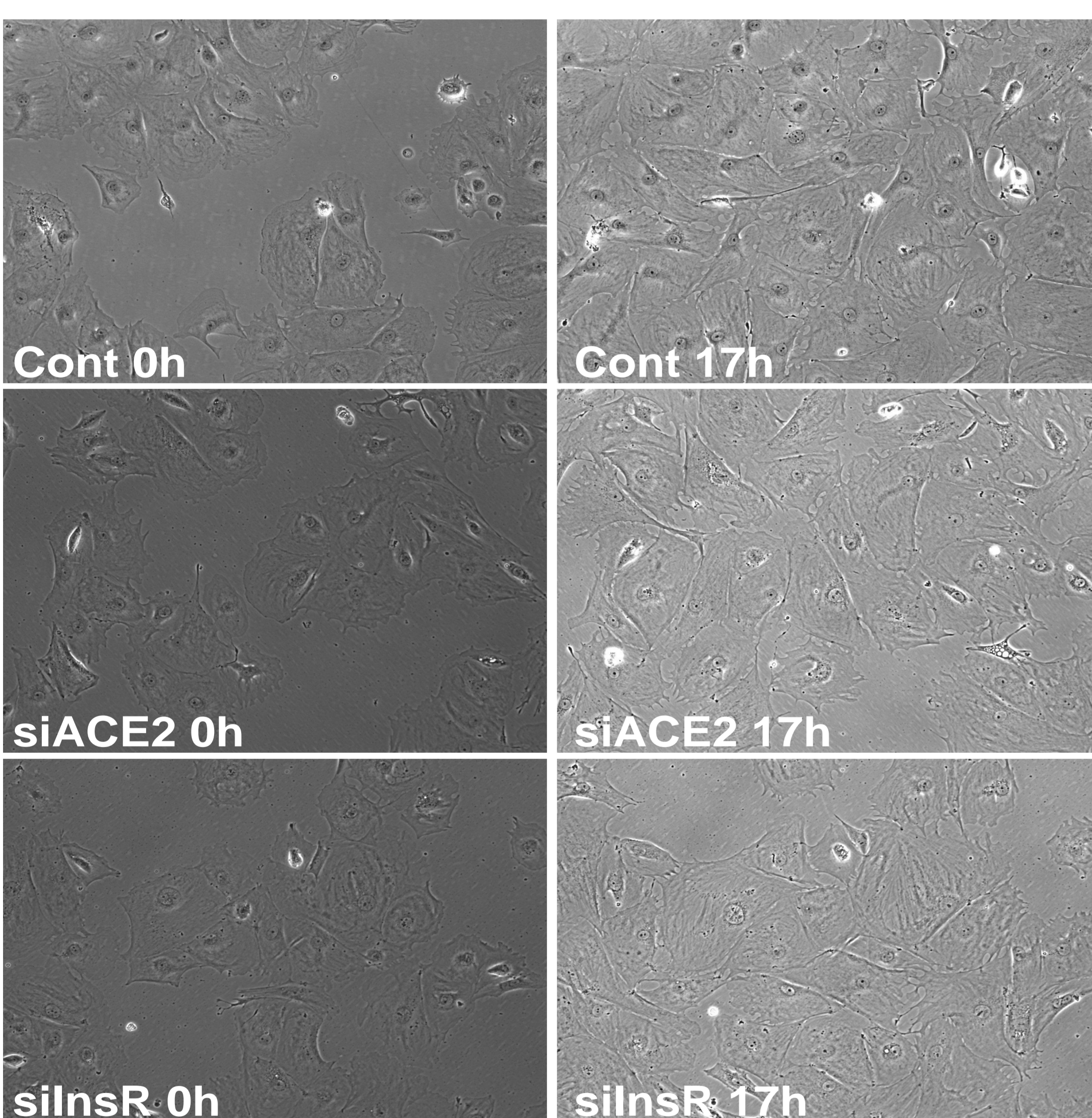


Figure 1. Podocyte motility. After 10 days of differentiation, podocytes were transfected with control siRNA (Cont), ACE2 siRNA (siACE2) or InsR siRNA (siInsR). After 24h of transfection, cells were kept for 17 hours. One photo was taken every hour in the Zeiss Cell Observer.

Representative images of the 0 and 17h of incubation are shown. Clear changes were observed between silenced and non-silenced cells after 17h of recording.

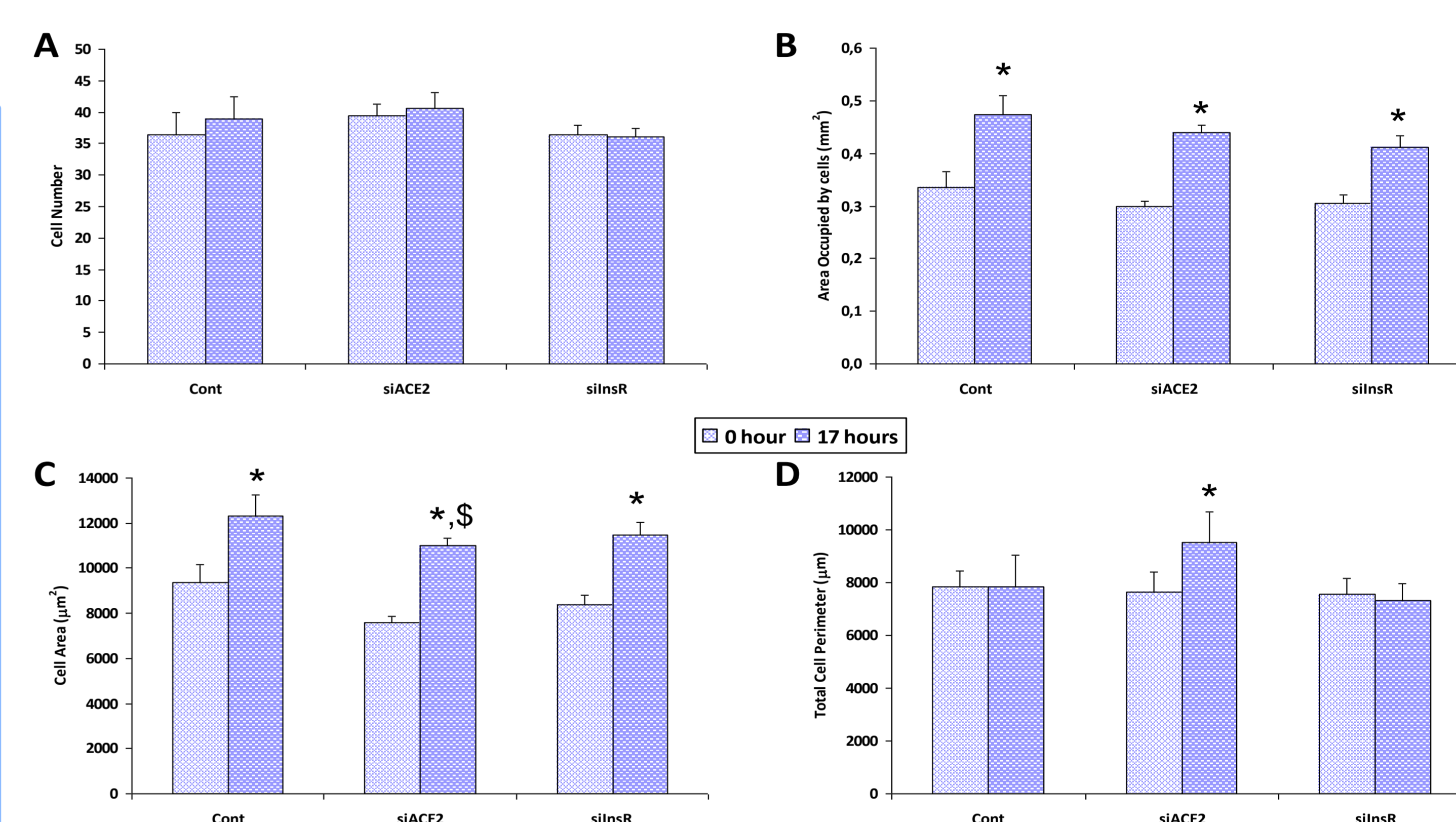
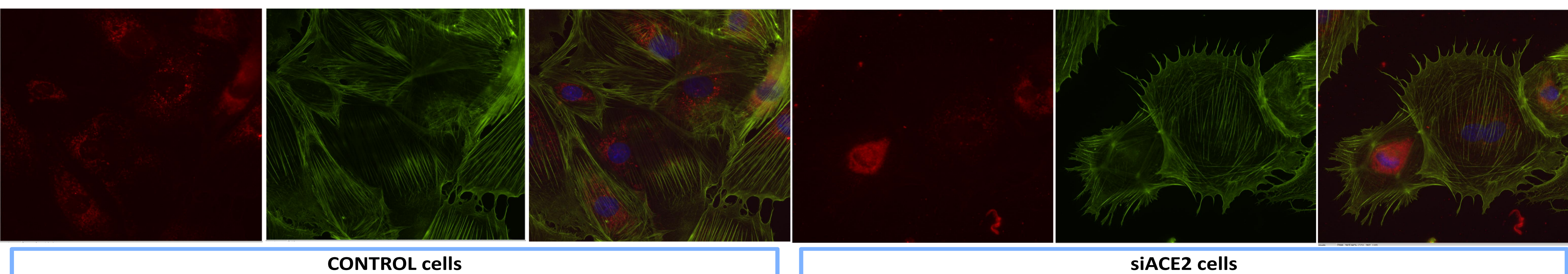


Figure 2. Changes from 0 to 17 h. From the captured images, Image J software was used to measure mean of cell number per field (A), mean of area occupied by the cells in one field (B), mean of cellular area (C), mean of total cellular perimeter (D). Cells with lower expression of ACE2 showed smaller area per cell as compared to control ones indicating different expansion capability when ACE2 is not expressed. * $p < 0.05$ vs. Cont_0h; \$ $p < 0.05$ vs. Cont_17h

Figure 3. ACE2 and F-actin staining. After 48h of silencing, cells were stained for ACE2 (red) and F-actin (green). ACE2 staining was decreased in ACE2-silenced cells. Furthermore, F-actin showed the differential cytoskeleton rearrangement



CONCLUSIONS

- ACE2 may help to keep the shape and integrity of podocyte cytoskeleton.
- In diabetic nephropathy, RAS imbalance is modified and ACE2 loss may contribute to cell rearrangements in the podocyte, aggravating the disease.

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