Lamin A/C in renal tubular cells is important for calcium oxalate crystal adhesion, tissue repair and cell proliferation, and associated with other potential calcium oxalate crystal receptors

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Introduction

Previous expression study has indicated that lamin A/C (LMNA) level was increased in renal tubular cells in response to calcium oxalate monohydrate (COM) crystal adhesion. However, its functional significance in kidney stone disease remained unknown. In the present study, we performed functional analyses of LMNA to define its roles in kidney stone disease.

Materials and Methods

MDCK cells

Incubation with or without COM crystals (100 μg/ml) for 48 h

Expression validation of LMNA and nesprin-1 levels

- Western blot analysis
- Immunofluorescence staining

siRNA-mediated knock-down of LMNA expression

Functional analyses

- Scratch assay
- Total cell count
- Cell death assay
- COM crystal-binding assay
- Protein network analysis

Results

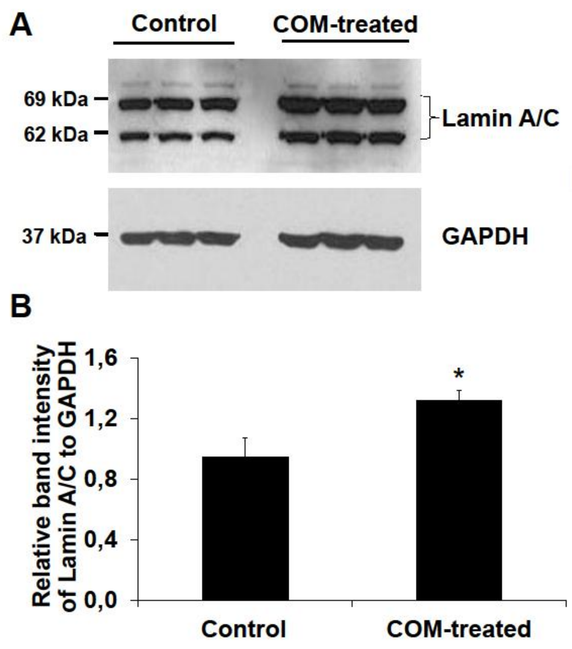

Figure 1. Confirmation of the increased levels of LMNA and its partner, nesprin-1, in COM-treated MDCK cells. (**A**) Western blot analysis for LMNA in MDCK whole cell lysate using mouse monoclonal anti-lamin A/C antibody. (**B**) Relative band intensity analysis of LMNA using GAPDH as the loading control (each bar was derived from 3 independent experiments; * = p<0.05 versus the control). (**C**) Immunofluorescence staining of nesprin-1 (stained in green using goat polyclonal anti-nesprin-1 antibody) and nuclei (stained in blue by Hoechst dye). Original magnification power was 400X.

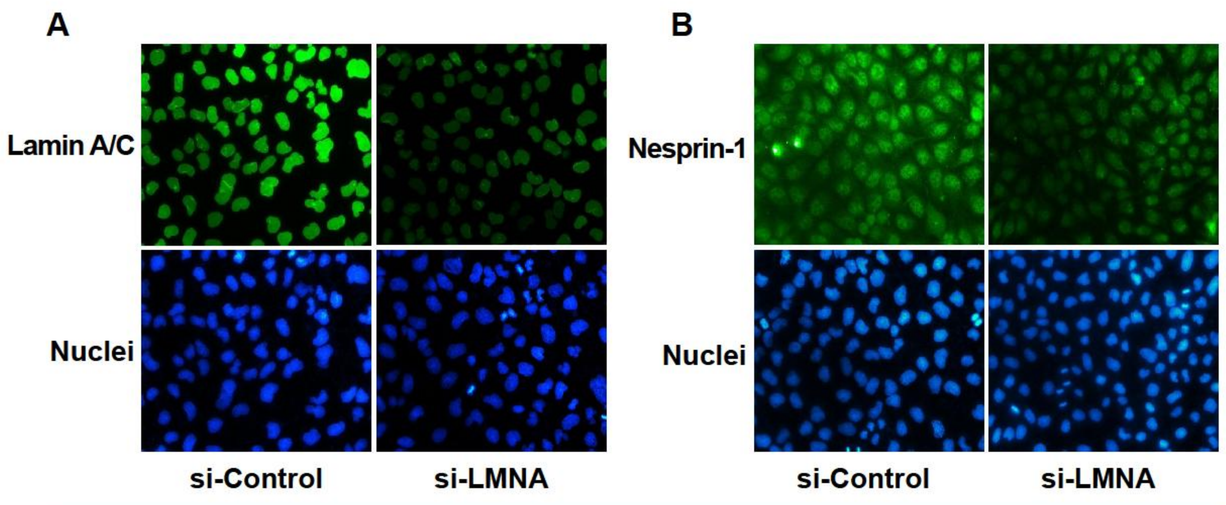

Figure 2. Confirmation of the efficiency of LMNA knock-down by si-RNA (A) and effect of si-LMNA on expression of nesprin-1 in MDCK cells (B). MDCK cells transfected with the controlled siRNA (si-Control) or siRNA specific to LMNA (si-LMNA) were grown on cover slips and stained using mouse monoclonal anti-lamin A/C or goat polyclonal anti-nesprin-1 antibodies, respectively. Both of these proteins were stained in green, whereas nuclei were stained in blue using Hoechst dye. Original magnification power was 200X.

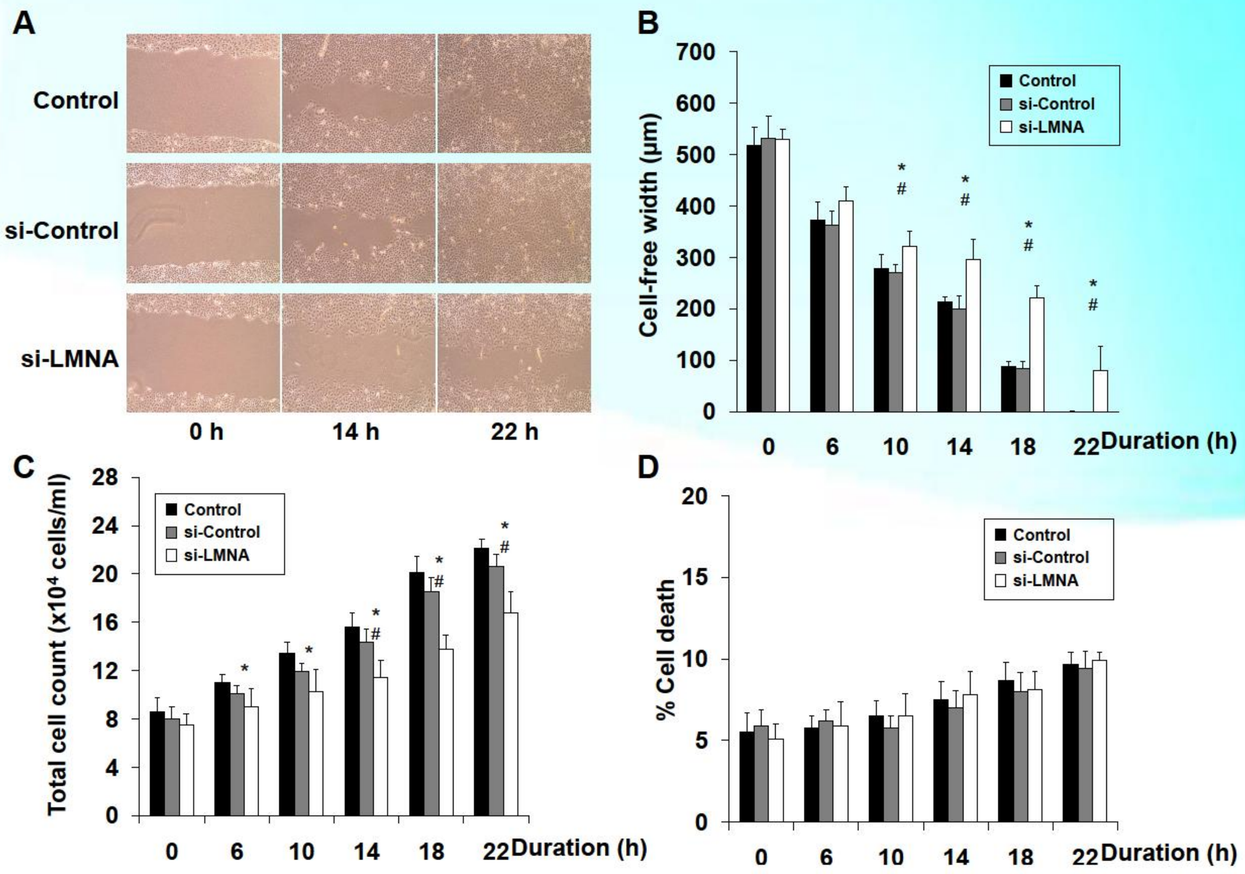

Figure 3. Effect of si-LMNA on tissue repair, cell proliferation and cell death. (**A**) Effect of si-LMNA on tissue repair was addressed using scratch assay. The cells without transfection (control) and those with si-Control served as the controlled conditions. Original magnification power was 100X. (**B**) The cell-free width was measured and analyzed by Tarosoft Image framework V.0.9.6 software. (**C**) Cell proliferation was addressed by total cell count after trypsinization. (**D**) Cell death was quantitated by Trypan blue staining. Each bar was derived from 3 independent experiments; * = p<0.05 versus control, # = p<0.05 versus si-Control.

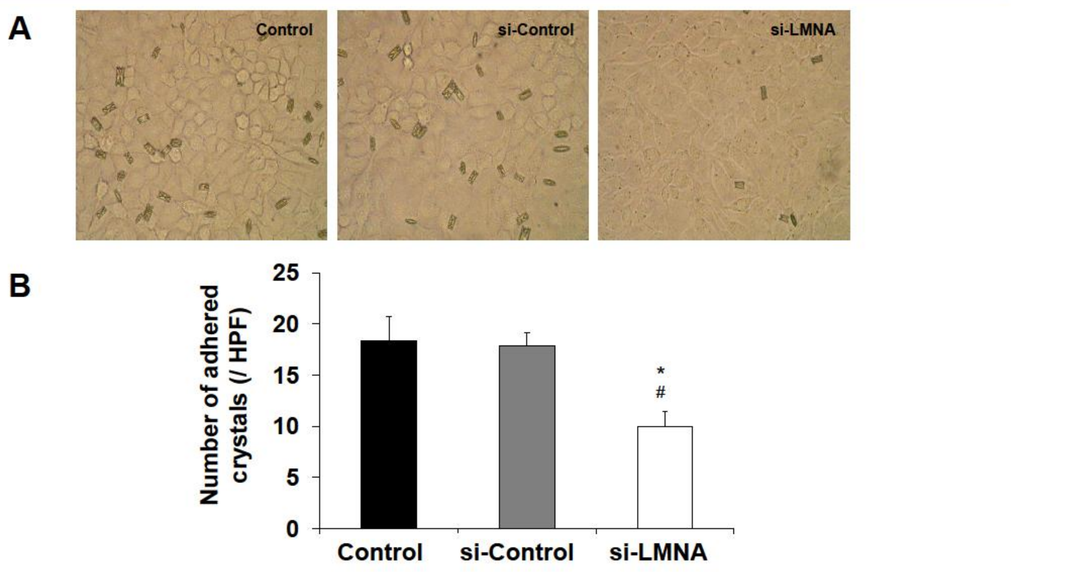

Figure 4. Effect of si-LMNA on COM crystal-binding capacity. COM crystal-binding assay was performed to examine crystal-binding capacity of controlled, si-Control-transfected, and si-LMNA-transfected cells. (**A**) Morphological examination using phase contrast microscopy. Original magnification power was 200X. (**B**) Quantitative analysis of the total number of COM crystals adhered on the cells after extensive washes. Each bar was derived from 3 independent experiments. HPF=high-power field; * = p<0.05 versus control, # = p<0.05 versus si-Control.

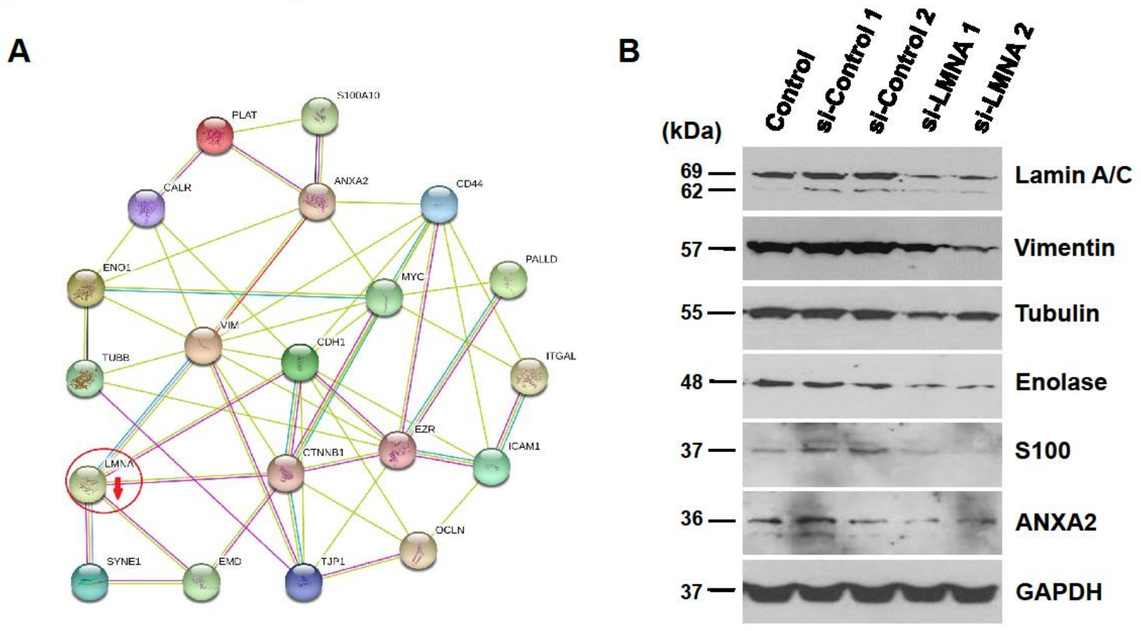

Figure 5. Associations of LMNA with potential COM crystal receptors. (**A**) Network analysis of LMNA with potential COM crystal receptors on apical membranes of MDCK cells. LMNA together with the potential receptors were submitted to STRING 9.0 tool. Each line represents a specific type of association between protein nodes. (**B**) Effects of si-LMNA on expression levels of these potential receptors as examined by Western blot analysis using GAPDH as the loading control.

Conclusion

These data have demonstrated for the first time that LMNA in renal tubular cells is important for COM crystal adhesion, tissue repair and cell proliferation, and associated with other potential receptors of COM crystals. Therefore, LMNA may serve as a potential target for prevention of kidney stone disease and its recurrence.

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L) Renal pathology. L1) Experimental pathology.
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