Roles of alpha-tubulin in renal tubular epithelial cell for cell viability, proliferation, tissue repair, and crystal adhesion in calcium oxalate kidney stone disease

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INTRODUCTION

Adhesion of calcium oxalate (CaOx) crystals on renal tubular epithelial cells is a crucial process that triggers many cascades of cellular response and alterations in protein expression. From our previous study, calcium oxalate caused changes in levels of a number of cellular proteins, including a decrease of tubulin. However, their functional significance remained largely unknown. In the present study, we aimed to address functional significance of tubulin in kidney stone disease.

MATERIALS AND METHODS

MDCK cell

- Control (transfected with empty vector)
- Alpha-tubulin overexpression (transfected with *pcDNA-TUBA1A*)

→ Incubate with or without CaOx crystals (1000 µg/mL) for 15 h

- Western blot analyses
- Cell death/viability assay
- Total cell count
- Scratch assay
- Crystal adhesion assay

RESULTS

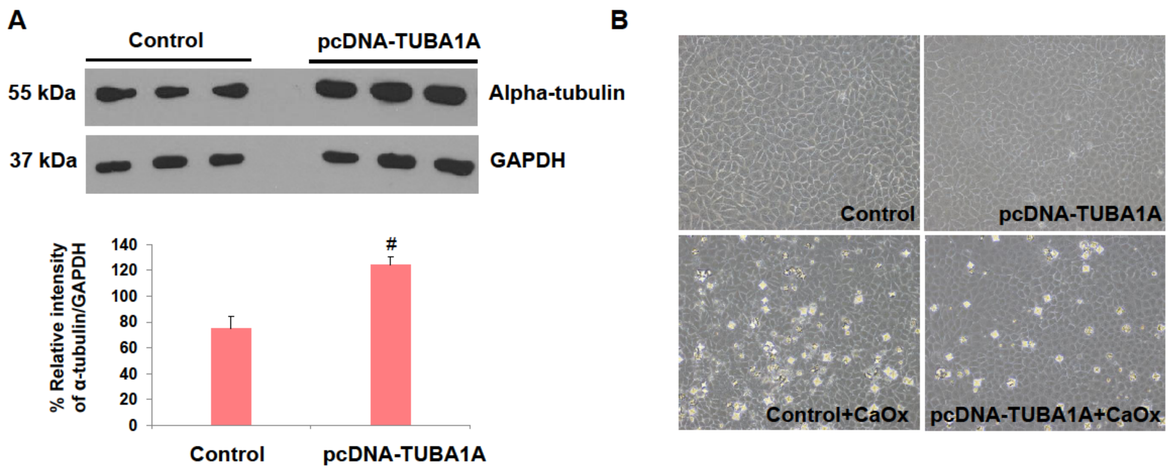

Figure 1. (A) Confirmation of alpha-tubulin overexpression by Western blotting. Relative band intensity data are presented as mean SEM (n=3). # = $p<0.05$ vs. control. **(B)** Morphology of MDCK cells.

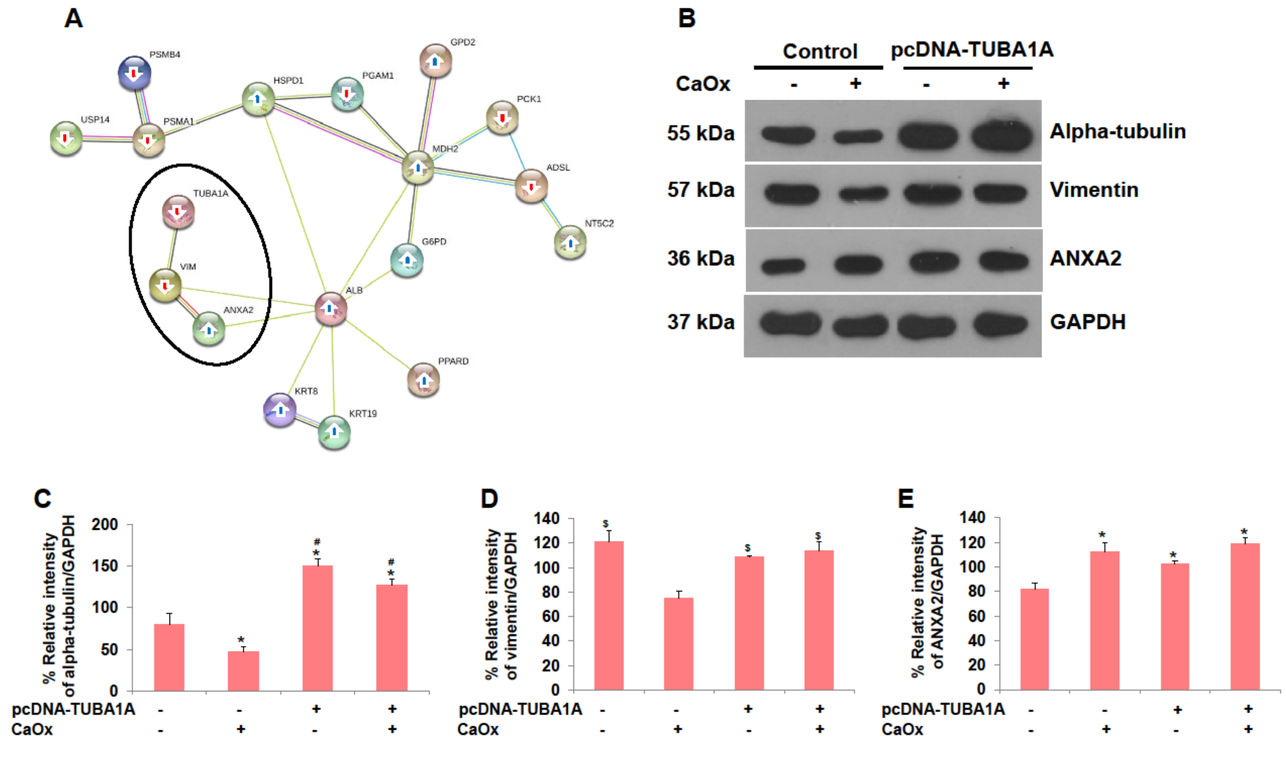

Figure 2. (A) Protein network analysis of alpha-tubulin and its associated proteins induced by CaOx crystals. **(B)** Western blot analysis of alpha-tubulin and its partners in whole cell lysate of MDCK cells. Band intensity of alpha-tubulin **(C)**, vimentin **(D)**, and ANXA2 **(E)** was normalized with GAPDH as the loading control. The data are reported as mean SEM (n=3). * = $p<0.05$ vs. control, # = $p<0.001$ vs. control+CaOx and $ = $p<0.01$ vs. control+CaOx

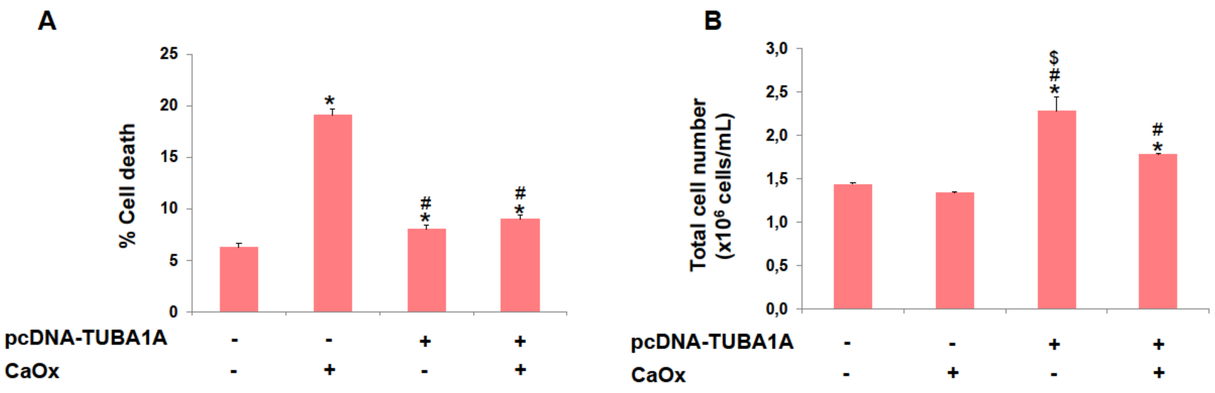

Figure 3. Effect of alpha-tubulin overexpression on cell death and cell proliferation. **(A)** Cell death was quantitated by trypan blue exclusion assay. **(B)** Cell proliferation was measured by total cell number after trypsinization. The data are reported as mean SEM (n=3). * = $p<0.05$ vs. control, # = $p<0.01$ vs. control+CaOx and $ = $p<0.01$ vs. pcDNA-TUBA1A+CaOx.

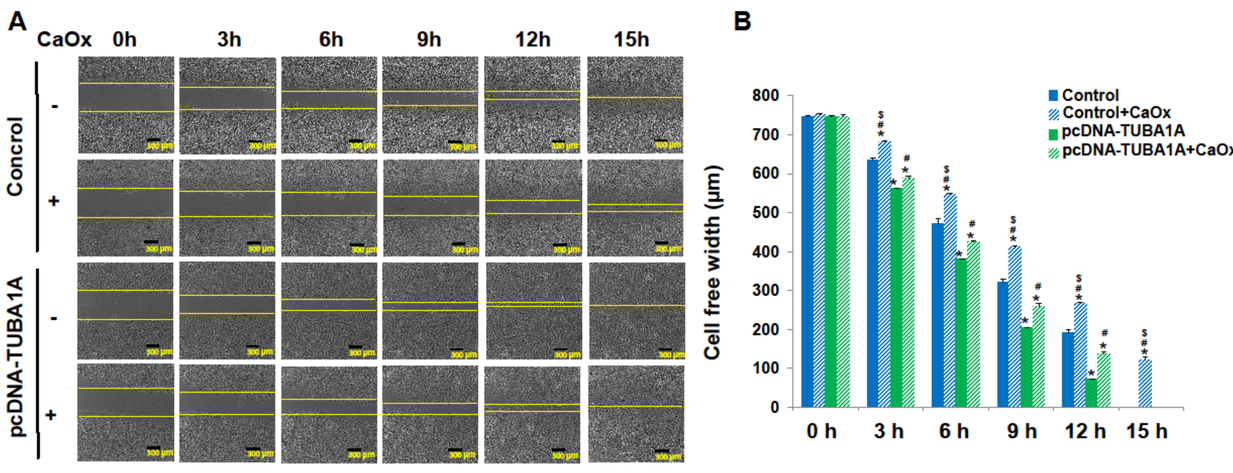

Figure 4. Effect of alpha-tubulin overexpression on tissue repair. **(A)** Evaluation of tissue repair was done by scratch assay. **(B)** Cell-free widths were measured at 0, 3, 6, 9, 12, and 15 h after scratching.using Tarasoft image framwork v.0.9.6 software. All the quantitative data are reported as mean SEM (n=3). * = $p<0.05$ vs. control, # = $p<0.05$ vs. pcDNA-TUBA1A and $ = $p<0.05$ vs. pcDNA-TUBA1A+CaOx.

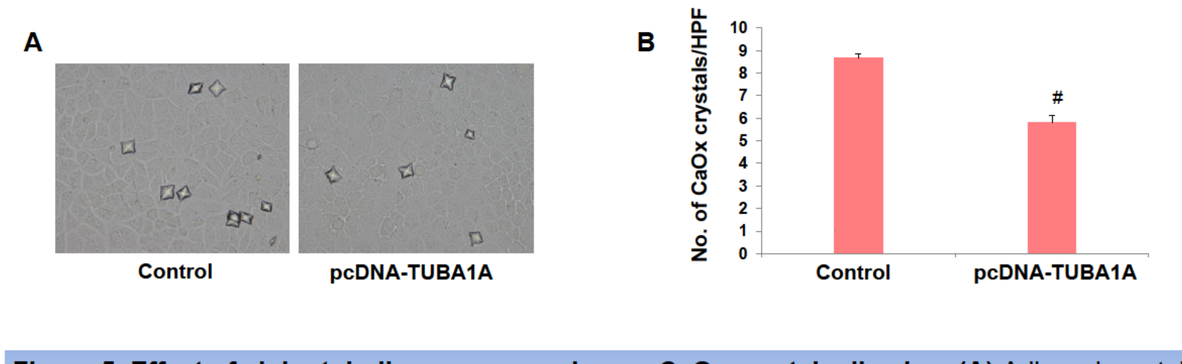

Figure 5. Effect of alpha-tubulin overexpression on CaOx crystal adhesion. **(A)** Adhered crystals were observed using a phase-contrast microscope. **(B)** Quantitative analysis of the total number of CaOx crystals on MDCK cells. The data are reported as mean SEM (n=6). * = $p<0.001$ vs. control.

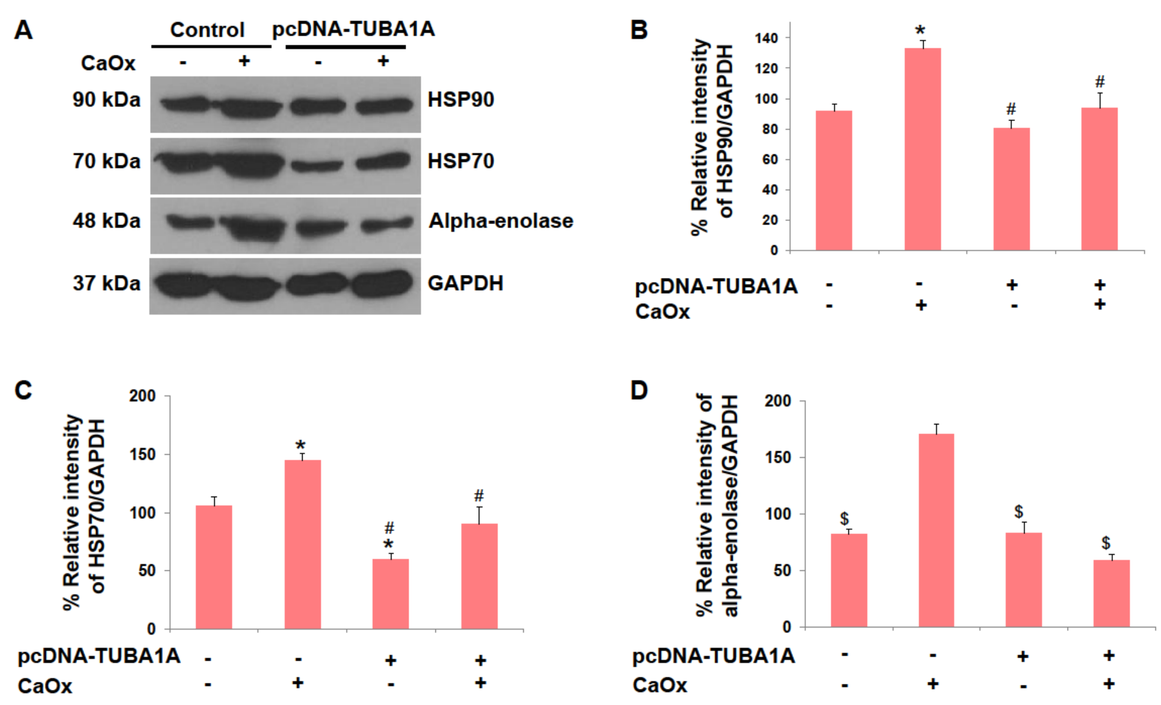

Figure 6. Effect of alpha-tubulin overexpression on crystal-binding proteins. **(A)** Western blot analysis of some potential crystal receptors in apical membrane of MDCK cells induced by CaOx crystals. Relative band intensity analysis of HSP90 **(B)**, HSP70 **(C)**, and alpha-enolase **(D)** using GAPDH as the loading control. The data are reported as mean SEM (n=3). * = $p<0.05$ vs. control, # = $p<0.05$ vs. control+CaOx and $ = $p<0.001$ vs. control+CaOx.

CONCLUSIONS

In summary, alpha-tubulin overexpression could prevent CaOx crystral-induced cytotoxicity, promote cell viability, proliferation and tissue repair, and attenuate crystal-cell adhesion by decreasing levels of potential crystal receptors. These findings implicate that alpha-tubulin may have protective roles in CaOx kidney stone disease, particularly at the crystal-cell adhesion step.

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E) Renal pathology. Experimental and clinical.
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