Talin-1 promotes Fibrosis in Clear Cell Renal Cell Carcinoma (ccRCC) through Renin-Angiotensin Signaling.

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Background

Fibrosis in the tumor microenvironment is a hallmark of solid tumors like clear cell renal carcinoma (ccRCC). The tumor microenvironment comprises extracellular matrix proteins and growth factors that promote cell proliferation and tumor growth. Increased extracellular matrix (ECM) deposition alters the cell surface receptor-mediated functions and their downstream targets to promote tumor growth. Integrins are heterodimeric cell membrane receptors crucial for organ development, structural maintenance, and disease pathologies of the kidney. Expression of various integrin subunits is increased in cancer. The complex between integrin and talin plays a major role in promoting fibrosis in cancer. Even though many of these basic cellular mechanisms by the cell surface receptor in the promotion of tumor growth have been understood for a while, an effective therapeutic inhibition has not yet been achieved. One reason for these failures is the complexity of interactions of membrane receptors with cytoplasmic proteins. Talin-1 is the major cytoplasmic protein that associates with integrin β1 and regulates its functions.

Method

Talin-1 downregulation in Caki-1 cells is achieved by shRNA-mediated downregulation. Cell proliferation, migration, focal adhesion, and spreading assays were performed using the KD talin-2 cells. The expression of the integrin-associated protein was investigated by immunoblotting.

Results

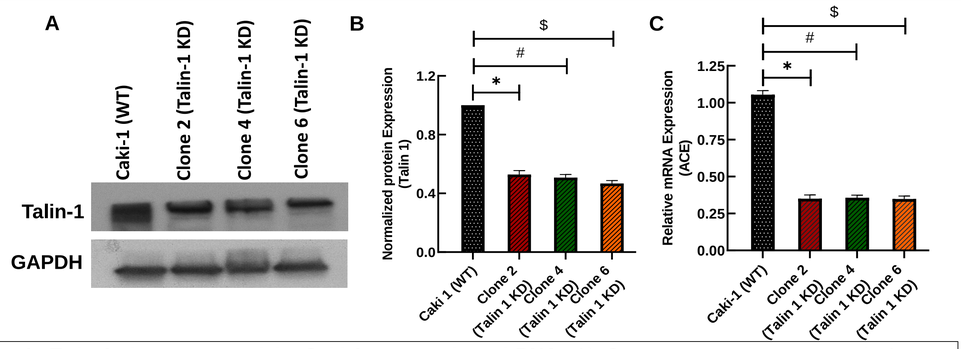

Fig. 1. Talin-1 downregulated in Caki-1 cells using shRNA. A) Clones expressing lower talin-1 were selected. Protein expression was determined by immunoblotting. C) qRT-PCR quantified mRNA levels of talin-1 show a significant decrease. *, #, $ p<0.05., n=3.

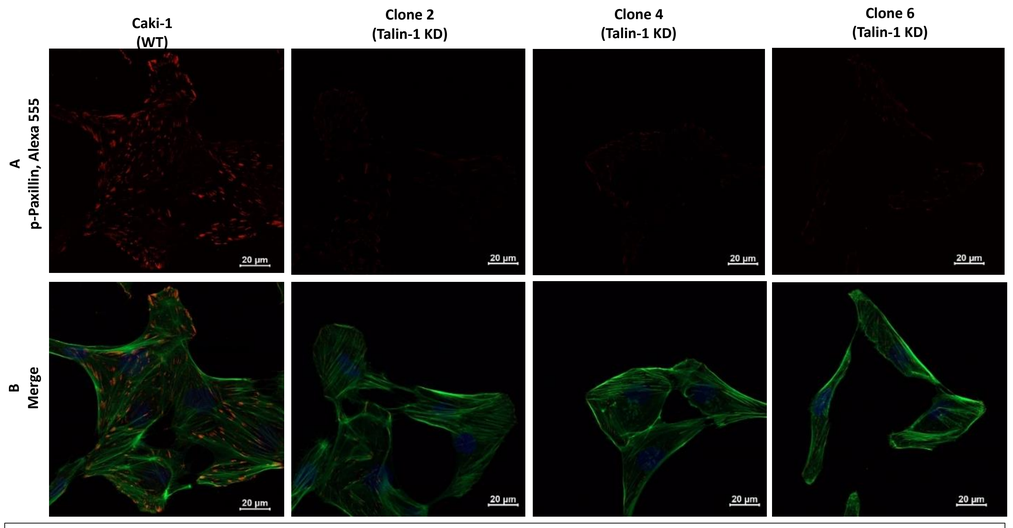

Fig. 2. Alteration in focal adhesion complexes,. (A) p-Paxillin antibody was visualized with Alexa Flour 555 (red); (B) Acton cytoskeleton was stained with phalloidin. Merged image. Scale 20 µm.

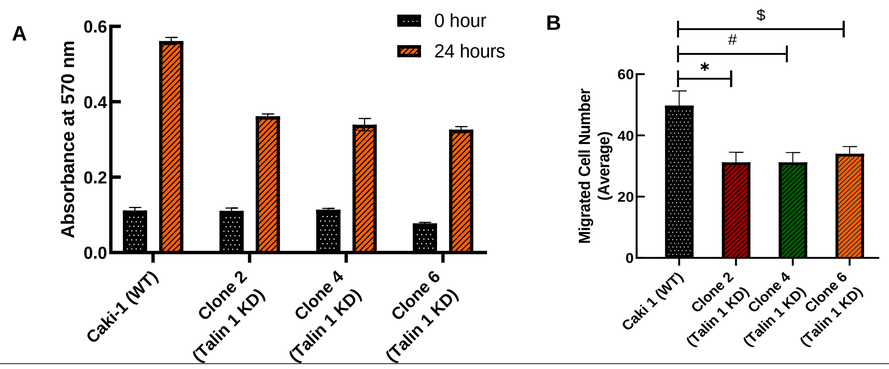

Fig. 3. Downregulated cell proliferation and migration in talin-1 knockdown, Caki-1 cells. (A) Reduced cell proliferation in talin-1 knockdown clones. (B) Decreased cell migration with the downregulation of talin-1 in Caki01 cells. n=3; *, #,$ p<0.05.

Results

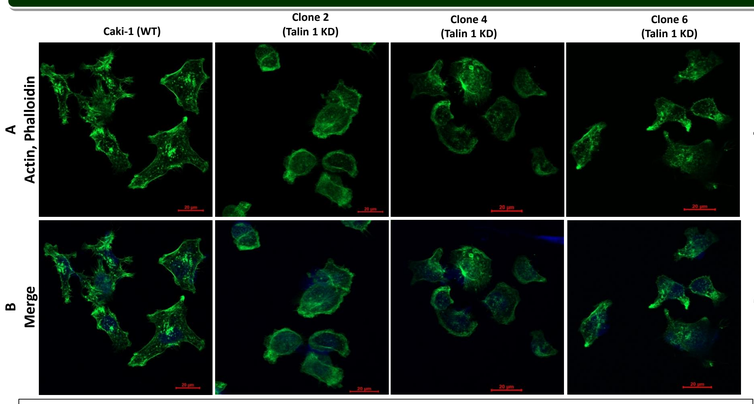

Fig. 4. Reduced cell spreading in collagen-1 with the knockdown of talin-1 in Caki-1 cells. (A) The actin cytoskeleton was stained with phalloidin. (B) Merged image of both DAPI and Phalloidin staining. Scale 20 µm.

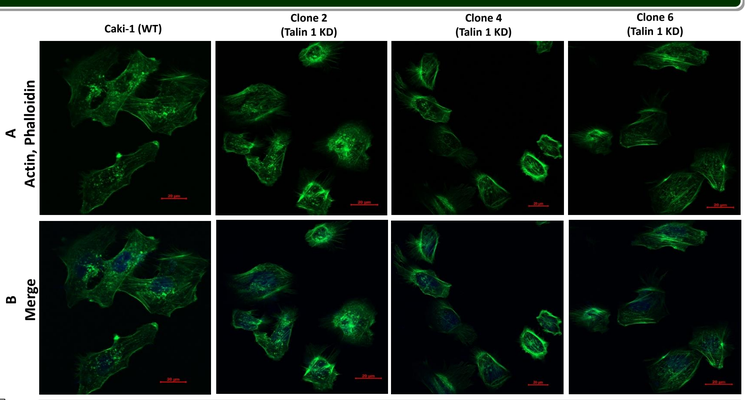

Fig. 5 Decreased cell spreading in vitronectin with the knockdown of talin-1 in Caki-1 cells. (A) The actin cytoskeleton was stained with phalloidin. (C) Merged image of both DAPI and Phalloidin staining. Scale 20 µm.

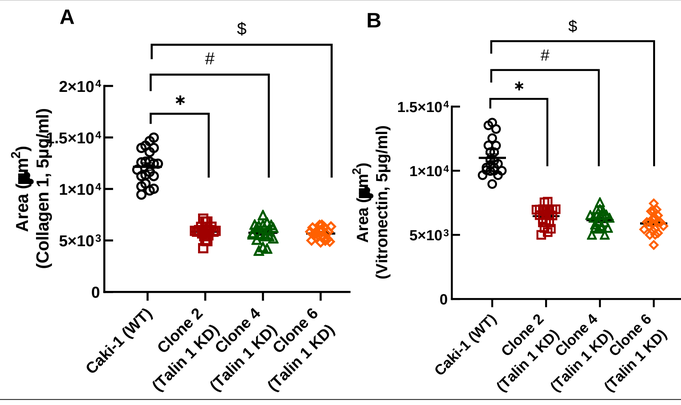

Fig. 7. Area (µm2) quantification shows a significant decrease in cell spreading with the KD of talin-1. (A) Reduced cell spreading area in collagen-1 with the downregulation of talin 1 in Caki-1. (B) The spreading area is significantly decreased in vitronectin with the knockdown of talin-1; n=30; *,#,$ p<0.05.

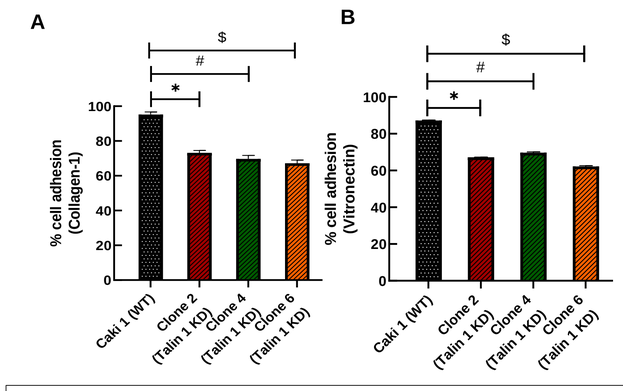

Fig. 8. Reduced cell adhesion with the knockdown of talin-1. (A) percent decreased cell adhesion in Collagen-1 in clones. (B) Decreased cell adhesion (%) in vitronectin with the downregulation of talin-1 (Clones) compared to the Caki-1 n= 6; *, #, $ p<0.05.

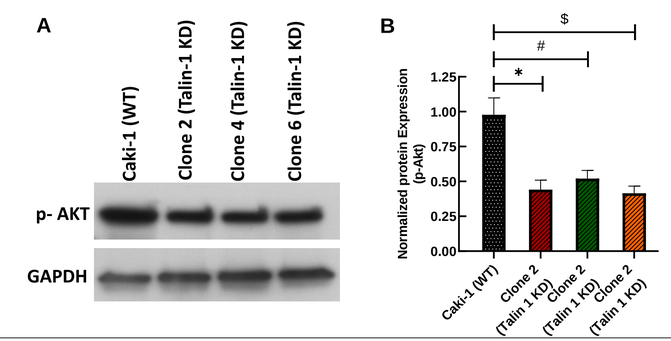

Fig. 9. Reduction in phosphorylation of AKT with the knockdown of talin-1 in Caki-1 cells. (A) Immunoblotting shows changes in the p-AKT levels in clones. (B) Normalized expression showed a significant decrease in phosphor-AKT with the knockdown of talin-1. *, #, $ p<0.05.

Fig. 10. Decrease in p-Erk1/2 levels in talin-1 KD cells. (A) Immunoblotting shows reduced p-Erk1/2 in clones compared to the wild type. (B) Normalized expression of phosphor-Erk1/2 shows a significant decrease with the knockdown of talin-1; *, #, $ p<0.05.

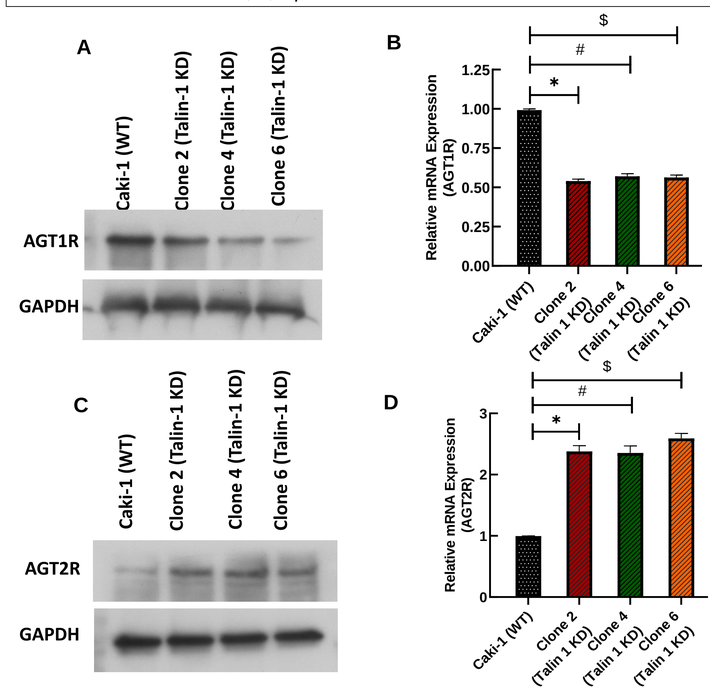

Fig. 11. Alteration in ang-2 receptors with the downregulation of talin-1 in renal cancer cells. (A) Immunoblotting for AGT1R in clones and Caki-1 wild type. ((C) Relative AGT1R mRNA expression shows a significant decrease in clones compare to Caki-1 (WT). (D) Immunoblotting for AGT2R using the cell lysate from clones and Caki-1. (E) Quantification of the immunoblotting shows a significant increase in AGT2R expression in clones. (F) Increase in AGT2R mRNA level with the KD of talin-1 in Caki-1 cells. * “WT vs Clone 2”, # “WT vs Clone 4, $ “WT vs Clone 6”; n=3, p<0.05.

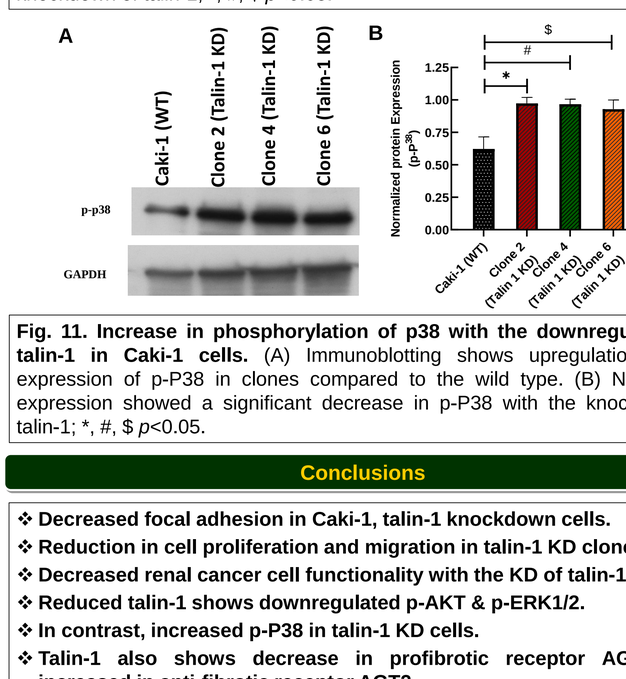

Fig. 11. Increase in phosphorylation of p38 with the downregulation of talin-1 in Caki-1 cells. (A) Immunoblotting shows upregulation in the expression of p-P38 in clones compared to the wild type. (B) Normalized expression showed a significant decrease in p-P38 with the knockdown of talin-1; *, #, $ p<0.05.

Conclusions

- **Decreased focal adhesion in Caki-1, talin-1 knockdown cells.**
- **Reduction in cell proliferation and migration in talin-1 KD clones.**
- **Decreased renal cancer cell functionality with the KD of talin-1.**
- **Reduced talin-1 shows downregulated p-AKT & p-ERK1/2.**
- **In contrast, increased p-P38 in talin-1 KD cells.**
- **Talin-1 also shows decrease in profibrotic receptor AGT1, and increased in anti-fibrotic receptor AGT2.**

Acknowledgments

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