

## INTRODUCTION

- Angiotensin II (Ang II) plays an important role in maintaining blood pressure homeostasis in health and diseases.
- Recent studies on Kidney cross-transplantation between wild-type and AT<sub>1a</sub> receptor-knockout (AT<sub>1a</sub>-KO) mice demonstrated a key role of kidney AT<sub>1a</sub> receptors in blood pressure control.
- However, Ang II receptors are widely expressed in intrarenal vasculature, proximal tubules, and renomedullary interstitial cells. The localization and mechanisms underlying the effect of kidney cross-transplantation on blood pressure has not been determined.
- The precise role of AT<sub>1a</sub> receptors in proximal tubules (PTs) in the regulation of arterial blood pressure is not fully understood.

## Construction of a proximal tubule-specific adenoviral GFP-tagged AT<sub>1a</sub> receptor or ECFP-tagged Ang II fusion protein vector

- Construction of a wild-type GFP-tagged AT<sub>1a</sub> receptor in a GFP-expressing vector (AT<sub>1a</sub>R/GFP) by Origene., or Ang II fusion protein in an ECFP-expressing vector (Dr. Julia Cook, Ochsner Clinic).

- Subclone the AT<sub>1a</sub>R/GFP gene or ECFP/All into a vector encoding a proximal tubule-specific sodium and glucose co-transporter 2 promoter (sglt2-AT<sub>1a</sub>R/GFP or sgl2-ECFP/All) by Vector BioLabs.

- Construction of an adenoviral vector encoding sgl2-AT<sub>1a</sub>R/GFP (Ad-sglt2-AT<sub>1a</sub>R/GFP) or Ad-sglt2-ECFP/All by Vector BioLabs (2.5 x 10<sup>11</sup> PFU/ml).



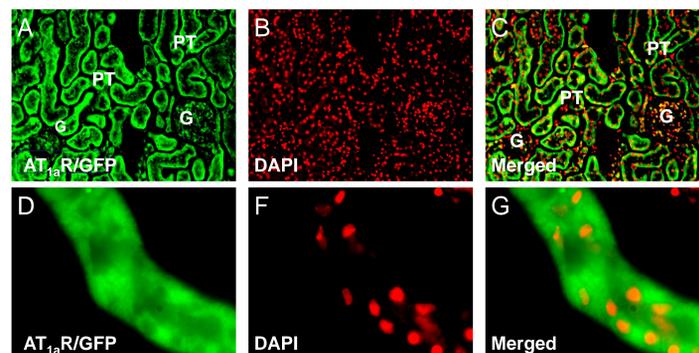
- I: Human Ad5-sequences (wt1-458); includes 5' L-ITR and packaging signal.
- II: transgene Sgl2-AT<sub>1a</sub>R/GFP-PolyA.
- III: Human Ad5 sequences (wt 3513-35935; E3 region deleted, includes 3' R-ITR. E3 deletion: nts 28587-30464).

## HYPOTHESIS

Proximal tubule-specific expression of AT<sub>1a</sub> receptors mediates both extracellular and intracellular Ang II-induced blood pressure responses in AT<sub>1a</sub>-KO mice.

## RESULTS

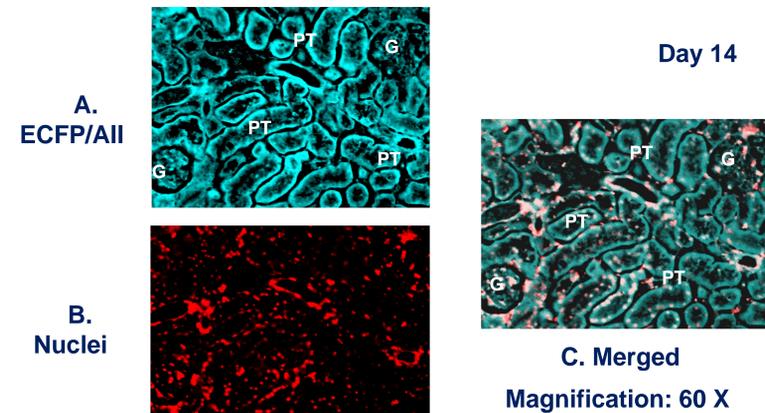
The sgl2 promoter drives the expression of GFP-tagged wild-type AT<sub>1a</sub> receptors selectively in proximal tubules of AT<sub>1a</sub>-KO mice



**Figure 1:** AT<sub>1a</sub>R/GFP is expressed primarily in proximal tubules, and there is little AT<sub>1a</sub>R/GFP expression in glomeruli. Magnification: 60 X (A-C); 200 X (D-G).

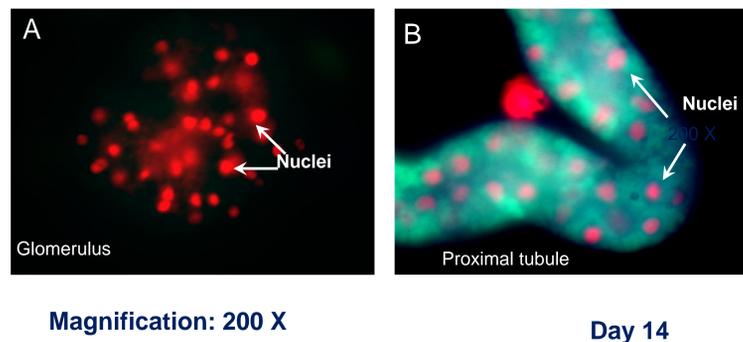
## RESULTS (Cont.)

The sgl2 promoter drives expression of ECFP/All selectively in proximal tubules of AT<sub>1a</sub>-KO mice



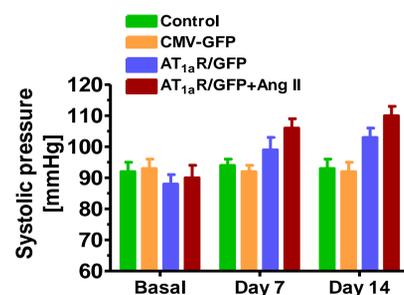
**Figure 2:** Panel A shows that ECFP/All is expressed in proximal tubules (PT). Panel B is DAPI-stained nuclei. Panel C is the merged image of Panels A and B. G = glomeruli.

Proximal tubule-specific expression of ECFP/All in AT<sub>1a</sub>-KO mouse kidney



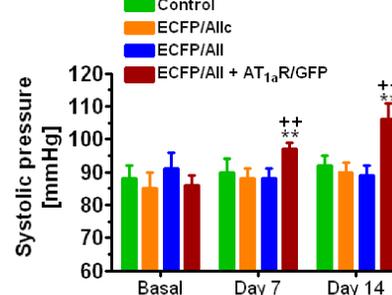
**Figure 3:** Panel A shows that ECFP/All is not expressed in freshly isolated glomeruli. Panel B shows that ECFP/All is expressed in freshly isolated proximal tubule.

Effects of proximal tubule-specific expression of AT<sub>1a</sub>R/GFP on blood pressure in AT<sub>1a</sub>-KO mice



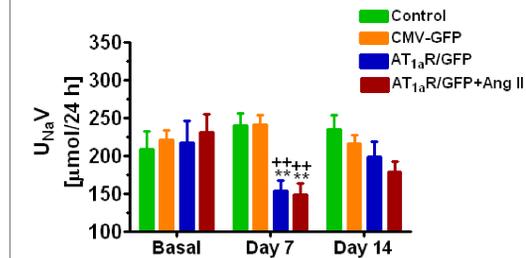
**Figure 4:** \*\*  $p < 0.05$  vs. basal; +  $p < 0.05$  vs. AT<sub>1a</sub>R/GFP. N = 10-12

Effects of proximal tubule-specific co-expression of ECFP/All and AT<sub>1a</sub> receptors on blood pressure in AT<sub>1a</sub>-KO mice



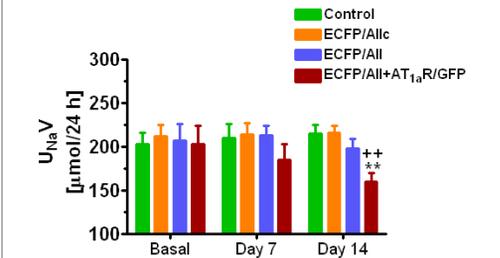
**Figure 5:** \*\*  $p < 0.01$  vs. basal; \*\*  $p < 0.01$  vs. control, ECFP/Allc or ECFP/All alone.

Effects of proximal tubule-specific expression of AT<sub>1a</sub> receptors on urinary sodium excretion in AT<sub>1a</sub>-KO mice



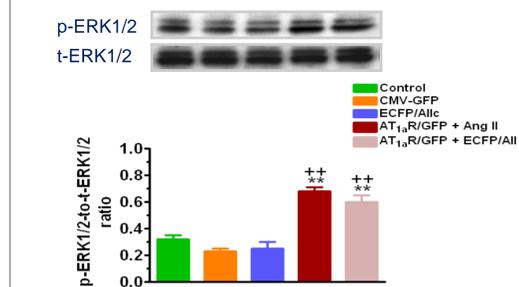
**Figure 6:** \*\*  $p < 0.01$  vs. basal; \*  $p < 0.01$  vs. control or CMV-GFP. N = 10-12

Effects of proximal tubule-specific co-expression of ECFP/All and AT<sub>1a</sub> receptors on urinary sodium excretion in AT<sub>1a</sub>-KO mice



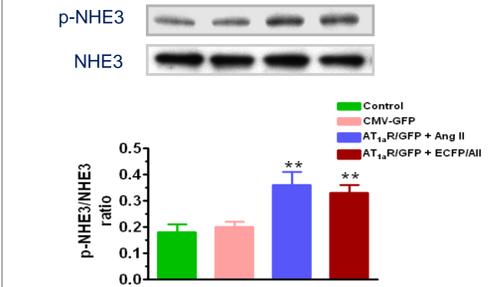
**Figure 7:** \*\*  $p < 0.01$  vs. basal; \*\*  $p < 0.01$  vs. control, ECFP/Allc or ECFP/All alone.

Effects of proximal tubule-specific co-expression of AT<sub>1a</sub> receptors and ECFP/All on phosphorylated ERK1/2 proteins in AT<sub>1a</sub>-KO mice



**Figure 8:** \*\*  $p < 0.01$  vs. basal; \*\*  $p < 0.01$  vs. CMV-GFP or ECFP/Allc alone.

Effects of proximal tubule-specific co-expression of AT<sub>1a</sub> receptors and ECFP/All on phosphorylated NHE3 proteins in AT<sub>1a</sub>-KO mice



**Figure 9:** \*\*  $p < 0.01$  vs. Control or CMV-GFP, n = 6-8

## SUMMARY

The sgl2 promoter selectively drove AT<sub>1a</sub>R/GFP or ECFP/All expression in proximal tubules (PT) of AT<sub>1a</sub>-KO mice.

PT-specific expression of AT<sub>1a</sub>R/GFP induced moderate increases in blood pressure.

PT-specific expression of ECFP/All increased blood pressure moderately only in the presence of AT<sub>1a</sub>R/GFP receptors.

The blood pressure responses to AT<sub>1a</sub>R/GFP receptor or ECFP/All expression were associated with moderate decreases in U<sub>Na</sub>V and increases in p-ERK1/2 and p-NHE3 proteins.

## CONCLUSION

Proximal tubule-specific expression of AT<sub>1a</sub> receptors in the kidney mediates extracellular and intracellular Ang II-induced blood pressure responses in AT<sub>1a</sub> receptor-knockout mice.

## ACKNOWLEDGEMENTS

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