Kidney Fibrosis And Acute Kidney Injury

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Abstract

INTRODUCTION AND AIMS: Male gender is a risk factor for the development of CKD and AKI, which suggests involvement of sex hormones. However, little is known about roles of androgen receptor signaling in kidneys under normal and pathological conditions. We aimed to address whether androgen receptor signaling contributes to the progression of kidney diseases.
METHODS: We used 9- to 11-weeks-old male androgen receptor-knockout (ARKO) mice on a C57BL/6 background (Sato T, et.al. Biochem Biophys Res Commun. 2003) and littermate wild type (WT) mice. We initially performed immunostaining using WT mouse kidneys to identify the localization of androgen receptors. To determine whether absence of androgen receptor signaling affects morphology and function of kidneys under normal condition, we carried out histological evaluation of ARKO mice kidneys and measured serum creatinine, urinary albumin, and kidney weight in ARKO mice. To investigate whether the deletion of androgen receptor alters the course of kidney diseases, we made 2 types of kidney disease models, unilateral ureteral obstruction (UUO) as a fibrosis model and ischemia-reperfusion injury (IR) after heminephrectomy as an AKI model, using ARKO and WT mice. The degree of fibrosis and levels of fibrosis markers in the kidneys were analyzed at 14 days after UUO in the first experiment, and the serum urea nitrogen levels were measured at 24 and 72 hours, and 7 days and 14 days after IR in the second experiment.
RESULTS: By immunohistochemistry, we identified androgen receptors in the nuclei of the tubular cells in the cortex of WT mice kidneys. Double-immunofluorescence revealed that androgen receptors were localized in proximal tubular cells stained with antibody to aquaporin 1. In phenotypic analysis of ARKO mice, we found that ARKO mice had significantly smaller kidneys than those of WT mice (WT 1.5 ± 0.1%, ARKO 1.2 ± 0.1%, shown as a ratio of total kidney weight to body weight, mean ± SD, $p<0.001$). ARKO mice kidneys were histologically normal, and serum creatinine and urinary albumin levels were not elevated in ARKO mice. In functional analysis using UUO model, we unexpectedly found by quantitative PCR that UUO kidneys of ARKO mice showed 2.0 fold increase in alpha smooth muscle actin (αSMA) mRNA and 2.3 fold increase in alpha-1 type I collagen mRNA levels than those of WT kidneys ($p<0.001$ and $p<0.05$, respectively), whereas no significant difference was observed in αSMA protein levels examined by western blotting or histological fibrosis scores. In another experiment, we found that blood urea nitrogen levels of ARKO mice at 24 hour after IR were significantly higher compared with WT mice against our prediction (ARKO 61.3 ± 17.7 mg/dL, WT 44.0 ± 11.9 mg/dL, shown as mean ± SD, $p<0.01$), although those were comparable at other time points.
CONCLUSIONS: Androgen receptors were expressed in proximal tubular cells. ARKO mice had smaller kidneys than WT mice with normal histology. ARKO mice kidneys subjected to UUO had higher mRNA levels of fibrosis markers compared with WT mice. Further, ARKO mice that underwent IR after heminephrectomy showed higher blood urea nitrogen levels at an early time point compared with WT mice. These results suggest that, contrary to our expectations, endogenous androgen receptor signaling plays protective roles in preventing kidney fibrosis and AKI.

Introduction and Aims

- Male gender is a risk factor for the development of CKD and AKI (Turin TC, et.al. J Am Soc Nephrol. 2012, Kummer D, et.al. Pediatr Nephrol. 2011), which suggests involvement of sex hormones.
- Little is known about roles of androgen receptor signaling in kidneys under normal and pathological conditions.
- We aimed to address whether androgen receptor signaling contributes to the progression of kidney diseases

Methods

- Localization of androgen receptors in adult wild-type mouse (WT) kidneys were determined by immunohistochemistry using rabbit anti-androgen receptor antibody (N-20, Santa Cruz Biotechnology, Santa Cruz, CA).
- Kidneys of 9- to 11-weeks-old male androgen receptor-knockout (ARKO) mice on a C57BL/6 background (Sato T, et.al. Biochem Biophys Res Commun. 2003) were histologically evaluated, and urinary albumin and kidney weight in the mice were measured to address whether elimination of androgen receptors affects kidneys under normal condition.
- ARKO and WT mice kidneys were subjected to unilateral ureteral obstruction (UUO) and mRNA levels of fibrosis markers were measured by quantitative PCR at 14 days after UUO to examine a role of androgen receptors in kidney fibrosis.
- ARKO and WT mice underwent Ischemia-reperfusion kidney injury (IR) after heminephrectomy and serum urea nitrogen levels were measured at 24 and 72 hours, and 7 days and 14 days after IR to investigate whether a lack of androgen receptors alters a course of AKI.

Results

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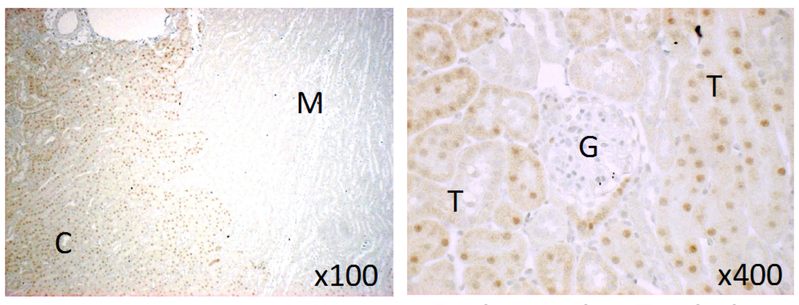
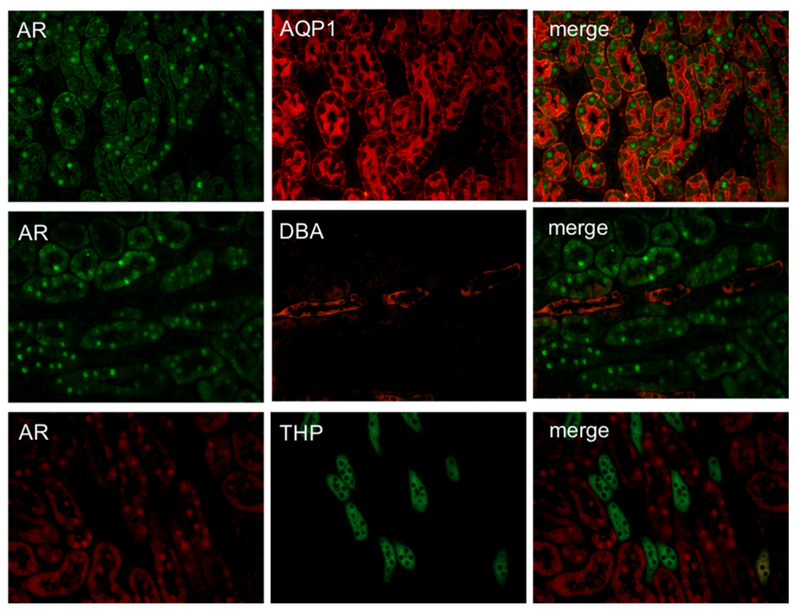


C: cortex, M: medulla　　G: glomerulus, T: tubules

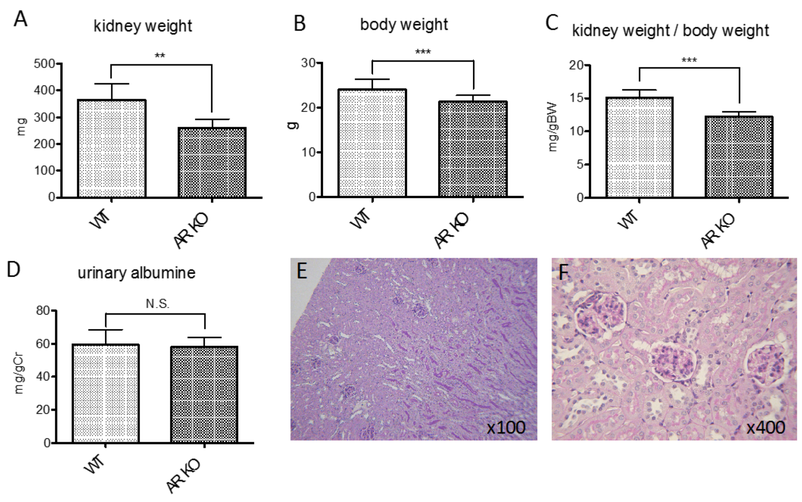
Imunostaining for androgen receptor (AR) using wild-type mice kidneys. AR was localized in nuclei of tubuler cells in the cortex. Right panel, Original magnification ×100 (left panel) and ×400 (right panel).

2



Double staining for androgen receptor (AR) and each tubler segmental marker. AR was localized in cells expressing proximal tubuler cell marker aquaporin 1 (AQP1) (upper panels). AR was not present in collecting duct stained with Dolichos Biflorus Agglutinin (DBA) (middle panels) or thick ascending limb of loop of Henle expressing tamm horsfall protein (THP) (lower panels) . Original magnification ×400.

3



Characterization of angrogen receptor knockout mouse (ARKO) kidneys under normal condition. (A, B) Kidney weight and body weight of ARKO were significantly lower than those of wild-type mouse (WT) . (C) Kidney weight was significantly lower in ARKO than WT even after adjusted to body weight. (D) Urinary albumin levels in ARKO were identical to those of WT. (E, F) Kidneys of ARKO were histologically normal. (A-D) Values are shown as mean ± SD. ***, $p<0.001$; **, $p<0.01$; N.S., $p>0.05$. (E, F) Periodic acid-Schiff stain. Original magnification x100 (E); x400 (F).

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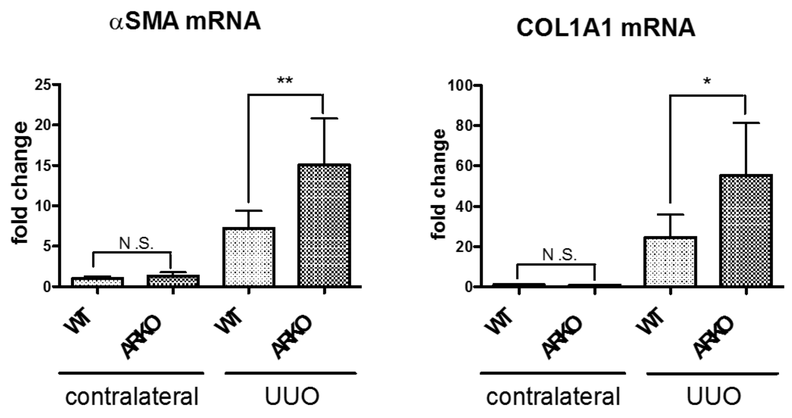

mRNA levels of fibrosis markers in androgen receptor knockout mice (ARKO) and wild type mice (WT) kidneys subjected to unilateral ureteral obstruction (UUO). αSMA and COL1A1 mRNA levels were higher in UUO kidneys from ARKO than those from WT. αSMA and COL1A1 mRNA levels were adjusted to those of GAPDH mRNA, and shown as fold change to mRNA of contralateral control kidneys from WT. Values are shown as fold change relative to WT contrarateral kidney. mean ± SD. **, $p<0.01$; *, $p<0.05$; N.C, $p>0.05$.

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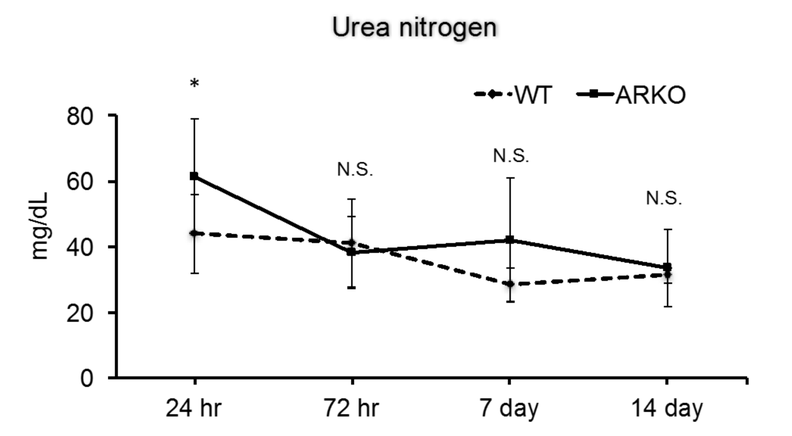

Serum urea nitrogen levels in androgen receptor knockout mice (ARKO) and wild type mice (WT) received ischemia-reperfusion injury (IR) after heminephrectomy. The urea nitrogen levels in ARKO was higher than those of WT at 24 hour, although the difference was diminished after 72 hour after IR. mean ± SD. *, $p<0.05$; N.C. , $p>0.05$.

Summary

- Androgen receptors were expressed in proximal tubular cells in mouse kidneys.
- ARKO mice had smaller kidneys than WT mice with normal histology.
- ARKO mice kidneys subjected to UUO had higher mRNA levels of fibrosis markers compared with those of WT mouse kidneys.
- ARKO mice that underwent IR after heminephrectomy showed higher blood urea nitrogen levels at an early time point than those of WT mice.

Conclusion

Endogenous androgen receptor signaling may play protective roles in preventing kidney fibrosis and AKI.

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