Characterization of P2rX7 knockout in PCK (Polycystic Kidney) rats

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INTRODUCTION

Accumulating evidence suggests that the autocrine and paracrine effects of Adenosine-3- phosphate (ATP) could be detrimental for the progression of polycystic kidney diseases (PKD). P2X7 targeting in cell cultures and zebrafish was shown to decrease cyst progression.

METHODS

In this project, CRISPR/Cas9 approach was employed to knockout P2X7 receptor in the PCK rat strain, a model of autosomal recessive PKD (ARPKD), to study involvement of P2X7 in PKD progression in mammals. Male PCK.P2rX7$^{+/+}$ and PCK.P2rX7$^{-/-}$ littermates were studied with H&E histomorhological evaluation of cyst progression, in-vivo renal function was evaluated with inulin clearance based glomerular filtration rate and measurements of 24hrs proteinuria. Because P2X7 receptors were found to regulate vascular tone we investigate possible effect of the knockout on blood pressure with implantable radiotelemetry. To evaluate epithelial electrolyte transport in cysts we applied patch-clamp electrophysiology to measure ENaC activity freshly isolated non-dilated ducts, early stage cysts and mature large cysts.

RESULTS

PCK.P2rX7$^{+/+}$ and PCK.P2rX7$^{-/-}$ animals exhibited moderate hypertension (blood pressure did not differ as registered with continuous DSI telemetry). Total P2X7 knockout causes exacerbated cystogenesis especially in cortex of adult rats compared to wild-type littermates. We observe insignificant proteinuria of in both groups (at 13 weeks) but with aging (at 24 weeks), it reached 89±13 and 157±19 mg/day/100g in wild-type and knockout animals, respectively. Glomerular filtration rate was also lower in the P2rx7$^{-/-}$ group (0.88±0.06 vs 1.22±0.11 ml/min/100g body weight) in young rats, however, with aging GFR significantly decreased in both groups and did not differ. We found that developing cysts exhibit increased ENaC activity whereas mature cysts had impaired channel activity (NPo= 0.78±0.1; 1.37±0.2 and 0.39±0.16). In all three tissue types P2X7 knockout increased ENaC activity (NPo=1.36±0.17; 2.3±0.36; 0.86±0.05), as expected.

CONCLUSION

P2X7 receptor contributes to protection against ARPKD development in PCK rats via limiting ENaC activity. Acknowledgement: K99/R00 HL116603, Baltimore PKD Center P&F Grant and MCW Gene Editing Rat Resource Center