Beta-3 adrenergic receptors as novel potential mediators of the cystogenetic process in ADPKD

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INTRODUCTION

Cyclic adenosine-3', 5'-mono-phosphate (cAMP) is a major driver of cyst growth. Vasopressin V2 receptors (V2R) are the main mediators of cAMP productions in the collecting ducts. Recent results revealed the presence of beta-3 adrenergic receptors (BAR3) in the kidney. BAR3 is found in the vasopressin sensitive segments of the nephron (thin ascending limb, thick ascending limb, distal convoluted tubule and cortical collecting duct) and are coupled to Gs proteins. BAR3 stimulation leads to an increase of intracellular cAMP. In addition, BAR3 stimulation induces Aquaporin 2 apical trafficking and Na-K-2Cl cotransporter NKCC2 activation. We wished to characterize the potential role of BAR3 in contributing to pathogenic cAMP accumulation and to assessing its possible utility as a therapeutic target.

METHODS

Murine cell lines either heterozygous ($Pkd1^{+/-}$) or homozygous ($Pkd1^{-/-}$) for the deletion of Pkd1 coding gene were used to generate stable clones expressing BAR3. $Pkd1^{+/-}$ and $Pkd1^{-/-}$ cell lines are able to form tubule-like structure or cysts respectively when grown in 3D culture, respectively. Upon seeding, cells were stimulated with the non-selective beta agonist, Isoproterenol, for 10 days. On the last day pictures were taken and cyst size was measured using ImageJ.

RESULTS

$Pkd1^{+/-}$ or $Pkd1^{-/-}$ cells stably expressing BAR3 were used to assess BAR3 potential involvement in cystogenesis. Upon seeding in 3D matrigel cells were stimulated either with Isoproterenol, a non-selective beta agonist, or with vehicle alone. Image analysis shows that after 10 days of treatment, $Pkd1^{-/-}$+BAR3 cells form larger cysts as compared to $Pkd1^{-/-}$ cells, suggesting that this effect is due to BAR3 presence. $Pkd1^{+/-}$ and $Pkd1^{+/-}$+BAR3 did not form cysts under any condition, as expected.

CONCLUSIONS

The recent findings that BAR3 are expressed in the kidney and that they are able to modulate cAMP levels might suggest a new potential drug target in the treatment of PKD. Our results provide the proof-of-principle that BAR3 stimulation can influence cystogenesis, most likely by altering cytosolic cAMP content. Our next step is to further test the potential of BAR3 as a therapeutic target both *in vitro* and *in vivo* by administration of selective antagonists in cell and mouse models respectively.