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P460 -Endogenous retinoic acid activity in collecting duct cells is a convergence point of regulation by AKI/CKD mediators

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Introduction: We previously reported that the canonical vitamin A signalling mediated by retinoic acids (RAs) and retinoic acid receptors (RARs) is physiologically confined to collecting ducts (CDs) in mouse kidneys; in mIMCD-3 mouse inner medullary CD cells, the RA/RAR signalling tightly controls the expression of many genes implicated in defence against infection, inflammation and fibrosis. This study aims to address whether (1) endogenous RA/RAR signalling is a common feature of cultured human and mouse CD cells, including CD-derived mesenchymal stem cells (MSCs) and CD-derived cystic cells from patients with autosomal recessive polycystic kidney disease (ARPKD); (2) key mediators of acute kidney injury (AKI) and chronic kidney disease (CKD) and neurotransmitters regulate RA/RAR signalling in CD cells; and (3) all-trans RA (tRA), a naturally occurring RAR pan-agonist, and RA-568, a synthetic selective RARα agonist, similarly restore RA/RAR signalling in CD cells, when repressed by AKI/CKD mediators.

Methods: The RA/RAR activities in human primary CD cells and age-matched CD-derived ARPKD cystic cells, human CD cell lines, mouse CD cell lines and a primary culture of Hoxb7 B2 mouse CD-derived MSCs were quantified by RA response element (RARE) dual-luciferase reporter assay. The endogenous RA/RAR activity was defined as the RARE-Luciferase activity repressed by both 4-diethylaminobenzaldehyde (DEAB), a RA synthesis inhibitor, and AGN193109, a specific RAR pan-antagonist. Effects of AKI/CKD mediators and neurotransmitters on RA/RAR activity were examined and efficacies of tRA and RA-568 in restoring any repressed RA/RAR activity were compared. One-Way ANOVA and t-test were used to compare multiple and two groups, respectively. $p<0.05$ was regarded statistically significant.

Results: Significant endogenous RA/RAR activities were observed in all cultures, human and mouse, primary and cell lines. In mIMCD-3 cells, RA/RAR activity was dose-dependently repressed by albumin, but not IgG or transferrin. Overexpression of Albumin, rather than the gene truncated of the sequence encoding its RA-binding domain, significantly repressed endogenous RA/RAR activities. Acetylcholine, aldosterone, angiotensin II, high glucose (25mM) and lipopolysaccharide dose-dependently reduced, while calcitonin gene-related peptide, endothelin-1, gentamicin, norepinephrine and vasopressin dose-dependently increased RA/RAR activity. Compared with aged-matched normal human CD cells, embryonic, perinatal and paediatric ARPKD cystic cells had significantly lower RA/RAR activity. Despite potency in normalising RA/RAR activity repressed by DEAB in CD cells, tRA was highly inefficient in rescuing RA/RAR activity repressed by albumin, high glucose, angiotensin II, aldosterone and lipopolysaccharide. In contrast, RA-568 was significantly more efficacious, in both mouse and human CD cells.

Conclusions: Our in-vitro data indicate that endogenous RA/RAR activity is a conserved feature of the CD cell lineage and appears to be a convergence point of regulation and dysregulation by AKI/CKD mediators and neurotransmitters. We propose that the RA/RAR signalling in CD cells may represent a novel target for intervention in AKI/CKD in order to improve AKI/CKD prognosis. Towards this end, the RARα agonist RA-568 might be more promising than tRA in restoring repressed RA/RAR signalling in CD cells. Further translational studies are clearly indicated.