

Canonical vitamin A signalling in collecting duct cells controls expression of anti-fibrotic microRNAs and is differentially regulated by AKI/CKD mediators

Background and aims: We previously reported that the canonical vitamin A signalling mediated by retinoic acids (RA) and retinoic acid receptors (RAR) 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 mRNAs that are involved in defence against infection, inflammation and fibrosis. This study aims to address whether (1) endogenous RA/RAR signalling is a common feature of human and mouse CD cells, including CD-derived mesenchymal stem cells (MSCs); (2) key mediators of acute kidney injury (AKI) and chronic kidney disease (CKD) regulate RA/RAR signalling in CD cells both *in vitro* and *in vivo*; and (3) endogenous RA/RAR controls microRNA (miRNA) expression in CD cells.

Methods: RA/RAR activities in mIMCD-3 cells, M-1 mouse cortical CD cells, mouse CD-derived MSCs and HCD human cortical CD cells were quantified by RA response element (RARE) dual-luciferase reporter assay. Endogenous RA/RAR activities were defined as *RARE-Luc* activities repressed by both 4-diethylaminobenzaldehyde (DEAB), a RA synthesis inhibitor, and AGN193109 (AGN), a specific RAR pan-antagonist. Effects of gentamicin, aristolochic acid, cisplatin, lipopolysaccharide (LPS), albumin (including albumin transgene), high glucose, aldosterone, angiotensin II, vasopressin, endothelin-1, calcitonin gene-related peptide (CGRP) on the endogenous RA/RAR activities were examined in mIMCD-3 cells and relation between albuminuria and RA/RAR activity in CDs was explored in Adriamycin-treated *RARE-Luc* transgenic mice. MiRNAs regulated by AGN, DEAB and albumin in mIMCD-3 cells were examined by microarray and Next Generation Sequencing (NGS).

Results: There were significant endogenous RA/RAR activities in mIMCD-3, M-1 and HCD cells, and mouse CD-derived MSCs. In these cells, endogenous RA/RAR activities were dose-dependently repressed by albumin ($p < 0.05-0.001$), but not IgG or transferrin; overexpression of the wild-type *Albumin*, rather than the gene truncated of the sequence encoding its RA-binding domain, significantly repressed endogenous RA/RAR activities ($p < 0.05-0.001$). In Adriamycin-treated *RARE-Luc* mice, heavy albuminuria is associated with significantly lower *RARE-Luc* activity in CDs. In mIMCD-3 cells, LPS, cisplatin, high glucose, angiotensin II and aldosterone dose-dependently reduced ($p < 0.05-0.001$), while gentamicin, aristolochic acid, vasopressin, endothelin-1 and CGRP dose-dependently increased RA/RAR activity ($p < 0.05-0.001$). Bioinformatic analysis of microarray data on RA/RAR-dependent mRNAs in mIMCD-3 cells suggested that endogenous RA/RARs could be anti-fibrotic by controlling miRNA expression; miRNA microarray analysis of AGN- and DEAB-treated mIMCD-3 cells suggested miR-29b, miR-30e and miR-140 to be potential RA/RAR targets. NGS unveiled heterogeneity in sequences of these miRNAs and confirmed two major forms of miR-140 were significantly downregulated by AGN, DEAB and albumin. Some miR-30, miR-29 family members and many other miRNAs were also significantly regulated by AGN, DEAB, and/or albumin.

Conclusions: Endogenous RA/RAR activity is a conserved feature of the CD cell lineage. AKI/CKD mediators differentially regulate RA/RAR activities in CD cells, suggesting different roles for these “risk factors” in AKI/CKD. Albumin repression of RA/RAR activity in CD cells requires its RA-binding domain and it explains why heavy albuminuria is associated with reduced RA/RAR activity in CDs. Finally, endogenous RA/RAR signalling regulates miRNAs in CD cells, including those with known anti-fibrotic activity, e.g. miR-140.