THE RESTITUTION OF AN OXALATE-DAMAGED EPITHELIUM

Sendyk D [1], Pescio LG [1-2], Fernández-Tomé MC [1-2], Casali CI [1-2].
[1]-Universidad de Buenos Aires, Facultad de Farmacia y Bioquímica-Biología Celular y Molecular. [2]- IQUIFIB- Instituto Dr Alejandro Paladini -UBA-CONICET.
E-mail: ccasali@ffyb.uba.ar

The renal inner medulla is responsible for the hydro-saline equilibrium maintenance through water and electrolyte excretion in urine. The collecting ducts, which are involved in the urine concentration, are immersed in an extracellular matrix with the highest body osmolarity. The hyperosmolarity is a key signal for cell differentiation and for the establishment of the urine concentration mechanism. Moreover, renal ducts are exposed to wastes coming from blood filtration. There are several nephrotoxic agents such as antibiotics, diuretics, antineoplastic and cytostatic agents, and renal stones. Calcium oxalate stones are the most common type of kidney stone. The crystal aggregates are harmful for epithelial renal cells and tubular structures, and that damage could lead to the development of chronic kidney disease. Our previous results showed that differentiated renal cells treated with oxalate (Ox) for 24 h lost the typical epithelial cobblestone morphology and showed a spindle-shaped morphology characteristic of an epithelial mesenchymal transition. After 48 h of Ox, cells started to recover their morphology and after 72 h of Ox the epithelium was almost reestablished. The aims of the present work were to evaluate whether epithelial integrity is disrupted after 24 h of Ox and whether epithelial differentiated characteristics are restituted after 72 h of Ox. To do that, the renal epithelial cells MDCK were grown in a hyperosmolar environment (512 mOsm/Kg H_2O) for 72 h to get a differentiated epithelium, and then subjected to 1.5 mM Ox for 24, 48 and 72 h. After treatments, cell morphology and the expression of differentiated epithelia markers were evaluated by fluorescence microscopy. E-cadherin, a member of adherens junctions, was localized to the cell periphery at 24, 48 and 72 h in control conditions. After 24 h of Ox, the protein was internalized and its label on the periphery decreased. After 48 h of Ox, E-cadherin was localized both to the cell membranes and to the cytoplasm, while after 72 h of Ox the label was mainly at the cell periphery. In control cells the apical marker gp135 was localized at apical cell surface, while in cells treated with 24 h of Ox gp135 apical staining was reduced. After 48 h of Ox, the percentage of cells expressing apical gp135 started to increase reaching values like control conditions at 72 h. Finally, primary cilium was evidenced by acetylated-tubulin immunofluorescence. Control cells showed a high percentage of ciliated cells, while it decreased upon treatment with 24 h of Ox. After 48 h of Ox, the cells started to recover the primary cilium, and after 72 h of Ox, the percentage of ciliated cells reached control values. The results showed that the treatment with 24 h of Ox induces dedifferentiation and after 72 h of the cell damage there is a restitution of the differentiated epithelia. The next goal is to elucidate the molecular mechanisms involved in the restitution of the oxalate-damaged epithelium.

CB-P05-56

G-QUADRUPLEXES AND CELLULAR NUCLEIC ACID BINDING PROTEIN (CNBP) MODULATE *TCOF1* (TREACHER COLLINS FRANCESCHETTI 1) TRANSCRIPTION

Gil Rosas, ML[1], Centola CL[1], Mouguelar VS[1], David AP[1], Piga E[1], Gomez-Zamorano D[2], Calcaterra NB[1], Armas P[1], Coux, G[1].
[1] IBR (CONICET/UNR), Facultad de Cs. Bioq. y Farm., UNR, Ocampo y Esmeralda, (2000) Rosario, Argentina. [2] Institut de Pharmacologie et Biologie Structurale, UMR5089 CNRS-Université de Toulouse, Toulouse, France.
E-mail: gilrosas@ibr-conicet.gov.ar

Treacher Collins Franceschetti Gene 1 (*TCOF1*) is involved in ribosomal RNA metabolism and is responsible for about 90% of mandibular dysostosis (MD) cases. Recently we identified a correlation in *TCOF1* and *CNBP* (cellular nucleic acid binding protein, a nucleic acids chaperone involved in rostral development) expression in human mesenchymal cells. As CNBP is a transcriptional regulator of several genes, we investigated the possible modulation of *TCOF1* expression by CNBP. Bioinformatic analysis yielded two CNBP consensus binding sites in *TCOF1* promoter (Hs-791 and Hs-2160). The sites coincide with G-quadruplex (G4, stable secondary structures formed by G-rich sequences that are built around tetrads of Hoogsteen-type hydrogen-bonded guanine bases) putative forming sequences (PQS). We confirmed *in vitro* that synthetic oligonucleotides containing these PQS folded into G4 by circular dichroism and intrinsic fluorescence. EMSA analysis with purified CNBP confirmed binding to the target G4s with Kd values in the nM range. Also, spectroscopic studies suggested that CNBP acted as a G4-unfolding protein over Hs-2160 G4. ChIP studies in HeLa cells extracts detected that CNBP was bound to Hs-791 and Hs-2160 sites in *TCOF1* promoter. HEK293 cell line expression studies revealed that Hs-2160 (but not Hs-791) PQS increased the transcription of luciferase controlled by the SV40 nonrelated promoter. Moreover, HEK293 cells treated with pyridostatin (a selective G4 stabilizing agent) showed increased endogenous *TCOF1* mRNA expression. In zebrafish *TCOF1* ortholog promoter we detected a site (Dr-2393) with equivalent properties to Hs-2160. G4 disruption in zebrafish embryos by microinjection of DNA oligonucleotides complementary to the G4 (antisense oligonucleotides or ASOs) resulted in decreased transcription of the *tcof1* gene and larvae with phenotypes compatible with *tcof1* knockdown. Finally, Morpholino-mediated *cnbp* knockdown in zebrafish induced *tcof1* expression. The results gathered here suggest that *TCOF1* transcriptional expression is modulated by CNBP through a mechanism involving G4 folding/unfolding. Also, that this regulation is active in vertebrates as distant as bonny fish and humans. These findings have implications in MD comprehension and treatment

CB-P06-48