Human Parietal Epithelial Cells (PECs) express ClC-5, megalin and cubilin *in vivo*

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Albumin uptake process at proximal tubular level is mainly driven by megalin, cubilin, and ClC-5. It was demonstrated the presence of these 3 proteins not only at tubular but also at glomerular level, in particular in podocytes. In addition, the ability of PECs to internalize albumin in mouse and rats *in vivo* and *in vitro* was observed. PECs can be identified by the expression of ANXA3. PECs expressing CD24, glycCD133 but negative for podocalyxin were classified as dysregulated parietal epithelial multipotent progenitors (APEMP) which have been supposed to be tubular progenitor cells. Nephrotic syndrome is the hallmark of both MCD and FSGS. The close clinical relationship between MCD and FSGS has suggested a shared pathogenesis but there are increasing evidences that makes this less likely.

Aims of this study were: 1) to verify the presence of ClC-5, megalin and cubilin in human PECs in control and proteinuric kidneys; 2) to define the phenotype of PECs expressing these tubular uptake machinery components; 3) to evaluate the expression of megalin, cubilin and ClC-5 at glomerular level in MCD and FSGS biopsies.

Serial section of 14 proteinuric kidneys and 8 control kidneys (pieces from site remote from tumor-bearing tissue histologically evaluated to disclose a normal morphology) were collected. All samples were immunostained for ClC-5 (Sigma-Aldrich), megalin (LS-Bio), and cubilin (R&D System). Representative patients were characterized for ANXA3 (Sigma-Aldrich), CD24 (Santa-Cruz Biotechnologies), and podocalyxin (Santa-Cruz Biotechnologies). Statistical analysis was performed using Mann-Whitney U-test and Spearmann's rank correlation test. $p<0.05$ was considered as significant.

PECs (ANXA3 positives) of control and proteinuric patients displayed the presence of megalin, cubilin and ClC-5. In particular, the positivity for the protein uptake system components was mainly located close to the tubular pole of the glomerulus. In serial sections of representative kidneys, PECs positive for ClC-5, megalin and cubilin were also positive for CD24 and negative for podocalyxin.

In both MCD and FSGS there was a decrease in ClC-5, megalin and cubilin positive glomeruli respect to controls (ClC-5 CTRL: 88.1%, MCD 11.1%, FSGS 30.8%) (Megalin CTRL: 59.3%, MCD: 24.3%, FSGS 43.1%) (Cubilin CTRL: 80.2%, MCD: 53%, FSGS: 31.1%), but only ClC-5 resulted to be significantly downregulated ($p<0.01$). Qualitative analysis revealed no differences in the expression of megalin and cubilin in podocytes of MCD and FSGS compared to controls, while ClC-5 was downregulated in these cells (CTRL: 94.6%, MCD: 33%, FSGS: 75%). PECs disclosed a huge decrease in positivity for megalin in MCD vs controls (CTRL: 46.3%, MCD: 10.6%), an increase in cubilin positivity in FSGS vs controls (CTRL: 19.4%, FSGS 30%), and no differences were observed in ClC-5 expression among control, MCD and FSGS PECs.

These preliminary data support the hypothesis that PECs expressing ClC-5, megalin and cubilin are APEMP, highlighting for the first time the existence of tubule committed progenitor cells among human PECs.

In addition, our results demonstrated a different expression pattern of ClC-5, megalin and cubilin between MCD and FSGS at glomerular level with a different involvement of podocytes and PECs, further underlining the pathophysiological differences of these two diseases.