Kidney progenitor cells in urine of cystinosis patients

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Background

Cystinosis is an inherited autosomal recessive lysosomal storage disorder characterized by the pathological accumulation and crystallization of cystine inside different cell types. If not treated, renal failure invariably develops within the first decade of life. We have quantified and characterized the kidney stem/progenitor cells (KSPC) voided in urine of patients and controls.

Materials and methods

We quantified the total number of cells and the number of undifferentiated kidney cells using qPCR analysis of RNA extracted from fresh urine samples of healthy donors (n = 9) and cystinosis patients (n = 14). The expression of the progenitor marker Vimentin was correlated to calibration curves derived from known numbers of adult kidney progenitor cells and normalized to volume and creatinine levels. Clonal KSPC lines were isolated and cultured from freshly voided urine of healthy volunteers (n = 11) and cystinosis patients (n = 6) and characterized by qPCR and FACS analysis.

Results (1) Quantification of urine-derived undifferentiated kidney cells

We demonstrated a significant increased loss of cells/creatinine in cystinosis urine (median = 920,78) compared to control urine (median = 5,13) and specifically a significant increase of stem/progenitor cells (Vimentin+) in cystinosis (median = 61,45) compared to controls (median = 0).

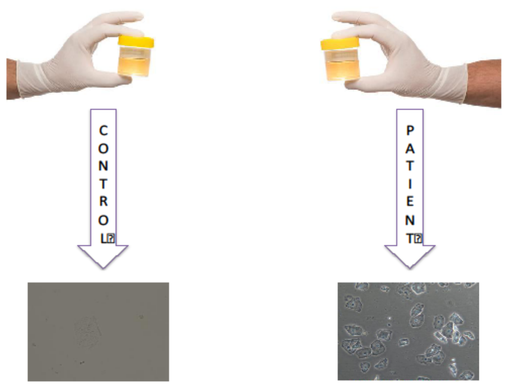

Figure 1. Comparison of quantification of vimentin+ cells in urine of control and cystinosis patients.

Results (2) Characterization of cystinosis urine-derived Undifferentiated cells

Cystinosis patients provided about 80 clonal progenitor cell lines per urinary sample while only 5 cell lines were developed from all control samples. Cystinosis cell lines presented mesenchymal phenotype and proliferated for more than 6 passages in culture, while control cell lines stopped growing at passage 3-4.

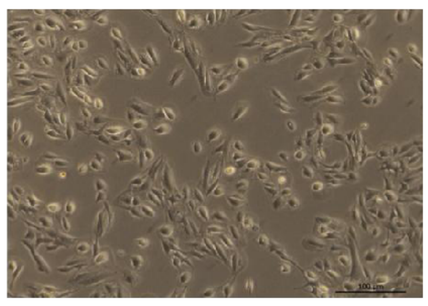

Figure 2. Representative image of cystinosis urinary clonal cell line at passage 4.

Results (3) Characterization of cystinosis urine-derived Undifferentiated cells

FACS analysis showed that the KSPCs isolated from cystinosis and control urine expressed mesenchymal stem cell (MSC) proteins as CD73, CD44, CD105, CD29, did not express hematopoietic stem cell (HSC) markers as CD34, CD45 and CD14 and were positive for the kidney progenitor proteins CD24 and CD133.

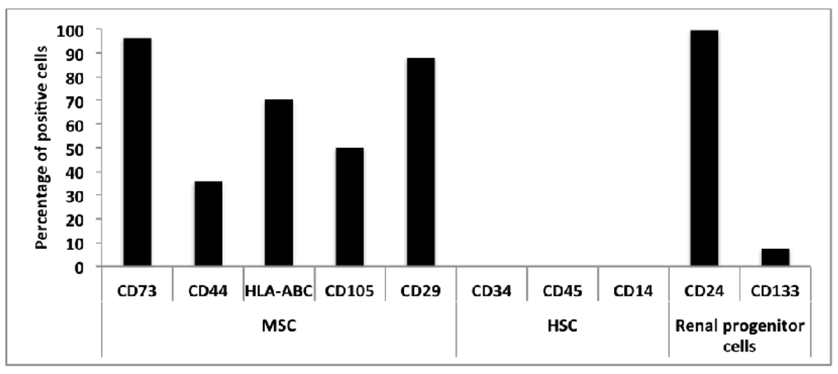

Figure 3. Representative panel of flow cytometry analysis of a cystinosis urine-derived clonal cell line.

Results (4) Characterization of cystinosis urine-derived Undifferentiated cells

Immunofluoresce staining showed the expression of the kidney stem/progenitor proteins CD133 and PAX2.

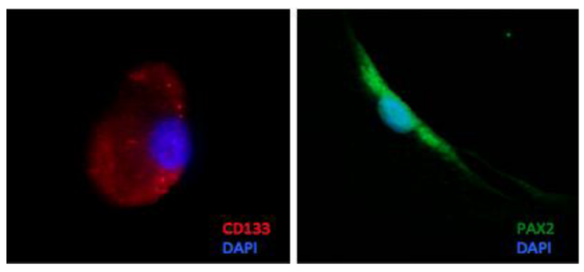

Figure 4. Representative images of immunostaining for cystinosis urinary cells showing expression of kidney progenitor markers CD133 (red) and PAX2 (green). Nuclear staining DAPI (blue). Magnification of 400X.

Discussion and Conclusions

Our data demonstrate increased loss of kidney stem/progenitor cells (Vimentin+ cells) in urine of cystinotic patients that might underlie the attempting for regeneration and a fast turnover of cells. Future studies will focus on potential of differentiation of the cells aiming their therapeutic use in regenerative medicine and possible correction of the cystinotic phenotype.

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