URINE AS SOURCE OF UNDIFFERENTIATED KIDNEY CELLS FOR PODOCYTE REPLACEMENT

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Background

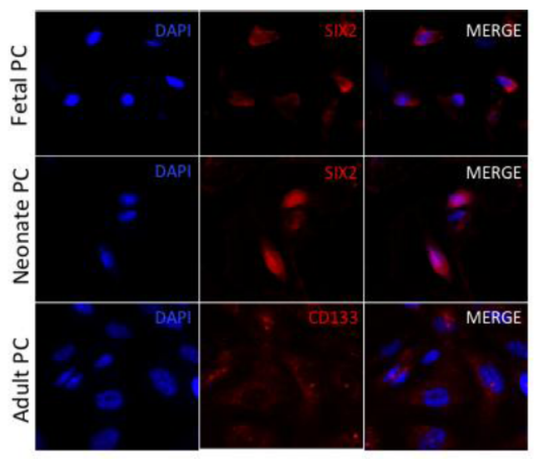
Loss of podocytes is the main cause of many glomerular diseases. Subpopulations of cells found in amniotic fluid (AF) and urine have been shown to express progenitor cell features and may have the potential to differentiate into several cell lineages. We aimed to obtain podocytes derived from kidney stem/progenitor cells (KSPC) isolated from (AF), principally composed of fetal urine, and freshly voided urine from neonates and adult donors.

Materials and methods

Fetal progenitor cells (fPC) were isolated from AF and urine progenitor cells were isolated from neonates (nPC) and healthy adult donors (aPC). Clonal cell lines were characterized as KSPC and KSPC-derived podocytes by gene expression analyses using quantitative PCR and protein expression by flow cytometry and immunofluorescence. Podocytes differentiation was induced by incubation of KSPCs in culture medium supplemented with retinoic acid and vitamin D. Functionality of podocytes was analyzed via albumin and calcium uptake.

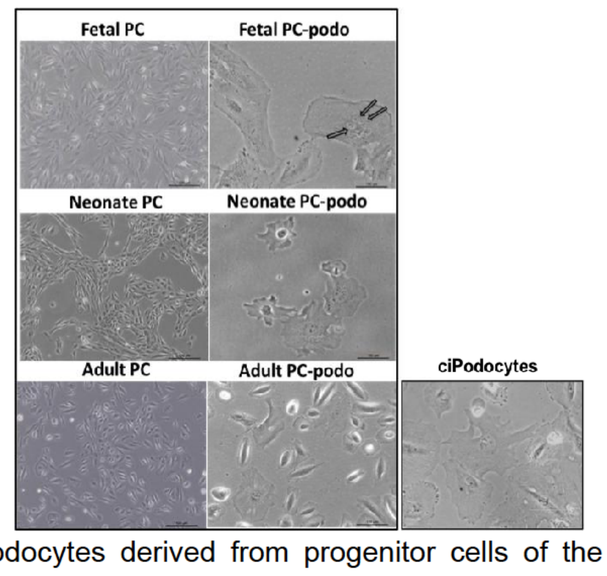
Results (1) *Kidney stem/progenitor cells characterization*

KSPCs expressed mesenchymal stem cell markers, but not hematopoietic markers. While fPCs and nPCs were positive for the fetal renal markers SIX2 and PAX2, aPCs expressed the adult renal epithelial markers CD133 and CD24.



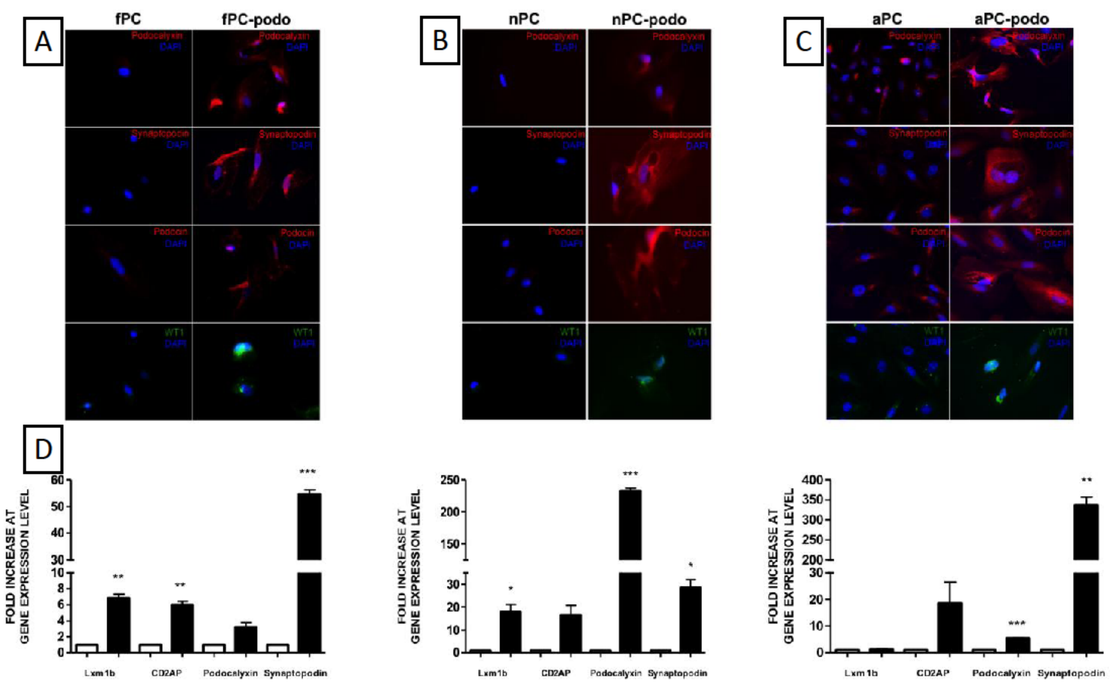
Renal progenitor markers. Immunofluorescence staining for the fetal renal marker SIX2 (positive in fetal and neonate PCs) and the adult renal epithelial marker CD133 (positive in adult PCs). Magnitude: 40X.

Results (2) *Podocytes derived from Kidney stem/progenitor cells*



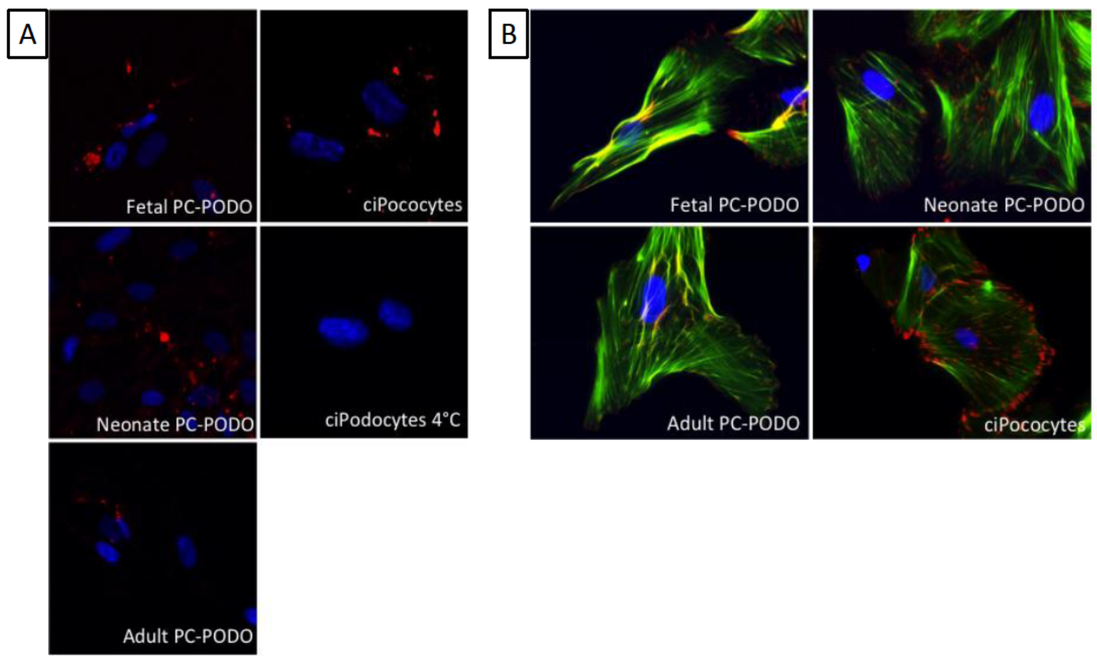
Cells morphology. Podocytes derived from progenitor cells of the 3 sources became bigger and many of them were bi- or multi-nucleated (arrows) with arborized cytoplasm as compared to fully differentiated ciPodocytes. Bright field images, Magnitude: Left side 5X, right side 20X.

Results (3) *Podocytes derived from Kidney stem/progenitor cells*



Protein and gene expression. A-C: Immunofluorescence staining for podocyte-specific markers: podocalyxin, synaptopodin, podocin and WT1. No expression was found in progenitor cells (a-c left panels), while podocytes derived from KSPCs expressed these specific markers (a-c right panels). Obj: 40X. **D:** Fold increase at gene expression level normalized to the expression in progenitor cells in qPCR. Note an up-regulation of podocyte-specific genes: Lmx1b, CD2AP, Podocalyxin and Synaptopodin on podocytes derived from KSPCs (*p<0,05; **p<0,005; ***p<0,0005).

Results (4) *Podocytes derived from kidney stem/progenitor cells*



Functionality of podocytes. **A:** Representative images of albumin uptake by podocytes derived of KSPCs, compared with ciPodocytes after 30 minutes of incubation at 37° C or 4° C used as control for endocytosis inhibition. Obj. 20X. **B:** Immunofluorescence staining of podocytes derived from KSPCs, presenting actin filaments (green) distribution and focal adhesion (red) similar to ciPodocytes. Magnitude. 40X.

Discussion and Conclusions

Different sources of stem cells have been used in animal studies to regenerate/repair kidneys in diverse renal injury models. Amniotic-fluid and urine-derived cells are promising sources of stem/progenitor cells collected non-invasively. **We demonstrate that some cell lines isolated from amniotic fluid, neonatal and adult urine present features of stem/progenitor cells and that these cells are committed to the renal linage, showing capacity to differentiate into functional podocytes *in vitro*. These cells may provide an important, non-invasive and novel source of cells for regenerative medicine aiming kidney repair.**

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