HIGH GLUCOSE DIFFERENTIALLY INDUCES GRADUAL LOSS OF PODOCYTIC MARKERS AND PARTIAL DE-DIFFERENTIATION

Nikolaos Tsotakos, Effie Tsilibary and Garyfalia Drossopoulou

Institute of Biosciences and Applications, National Centre for Scientific Research "Demokritos", Athens, Greece

BACKGROUND

Podocytes are visceral epithelial cells in the renal glomerulus which form the main permselective barrier for normal blood filtration. Their unique polarized phenotype, characterized by a main body with multiple primary and secondary processes, is maintained by means of expressing several podocyte-specific proteins, including podocalyxin and nephrin. Nephrin is a major scaffolding protein of the slit diaphragm, which takes part in transducing extracellular signals from the slit diaphragm to the intracellular actin cytoskeleton. Podocalyxin is the major sialomucin of the apical domain of foot processes, which is required for the formation and maintenance of foot processes. Loss of the negative charge of the podocytic glycocalyx, carried for the most part by podocalyxin, leads to phenotypic changes that have been associated with proteinuric diseases.

MATERIALS AND METHODS

We used an in vitro model of human glomerular epithelial cells (HGEC) to investigate the role of high glucose in dysregulating the podocytic epithelial phenotype and determined the time needed for this change to occur.

RESULTS

❖ **Vimentin is a well known mesenchymal marker and its upregulation is considered a significant marker of dedifferentiation and podocyte injury. Transient culture of HGEC in high glucose resulted in reversible upregulation of vimentin protein expression.** (Figure 1A, B).

❖ **CD10/CALLA is an important cell surface marker in the diagnosis of human acute lymphocytic leukemia and has long been considered a differentiation marker of renal epithelium. In vitro culturing of HGEC in the presence of high glucose levels resulted in permanent and irreversible downregulation of CD10/CALLA protein expression.** (Figure 1C, D).

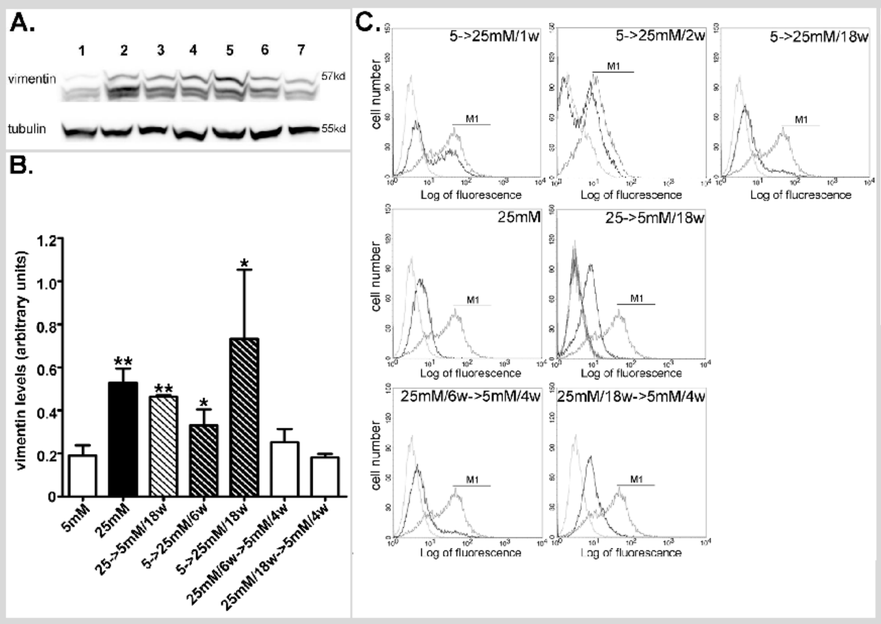

Figure 1: Vimentin and CD10/CALLA expression in podocytes. (A) Western blot analysis for vimentin expression in HGEC. Total protein was extracted from cells cultured in the presence of 5mM (control) (lane 1) or 25mM (lane 2) glucose. Lane 3 represents vimentin expression in HGEC continuously exposed to 25mM and reverted to 5mM glucose for 18 weeks (HGEC:25mM-to-5mM/18w). Lanes 4 and 5 represent HGEC continuously exposed to 5mM glucose and reverted to 25mM for 6 and 18 weeks respectively (HGEC:5mM-to-25mM/6w, HGEC:5mM-to-25mM/18w). Lanes 6 and 7 represent HGEC:25mM/6w-to-5mM/4w and HGEC:25mM/18w-to-5mM/4w respectively. For quantification of vimentin the 57kDa band was used. Blots were reprobed with anti-tubulin antibody, to verify protein loads, against which the data were normalized. (B) Results were expressed as the mean ± SD of three independent experiments. (C) Representative experiments from flow cytometric analysis of CD10/CALLA. Thick grey lines represent HGEC:5mM, thin grey lines represent the corresponding isotype control antibody and black lines represent stimuli. (D) Percentage of positive cells was calculated for the histogram section indicated as M1 (**p<0.01 vs. HGEC:5mM).

❖ **The slit diaphragm specific transmembrane protein nephrin, is implicated in the pathophysiology of proteinuria. In HGEC downregulation of nephrin occurred following 4 weeks of exposure to high glucose. This downregulation was concomitant with upregulation of the mesenchymal marker vimentin. Reverting glucose concentration to normal resulted in full restoration of total and cell-surface nephrin levels. (Figure 2). Therefore, the reversible phenotypic changes of expression in cultured podocytes are accompanied by normal levels of cell surface-associated nephrin. (Figure 2).**

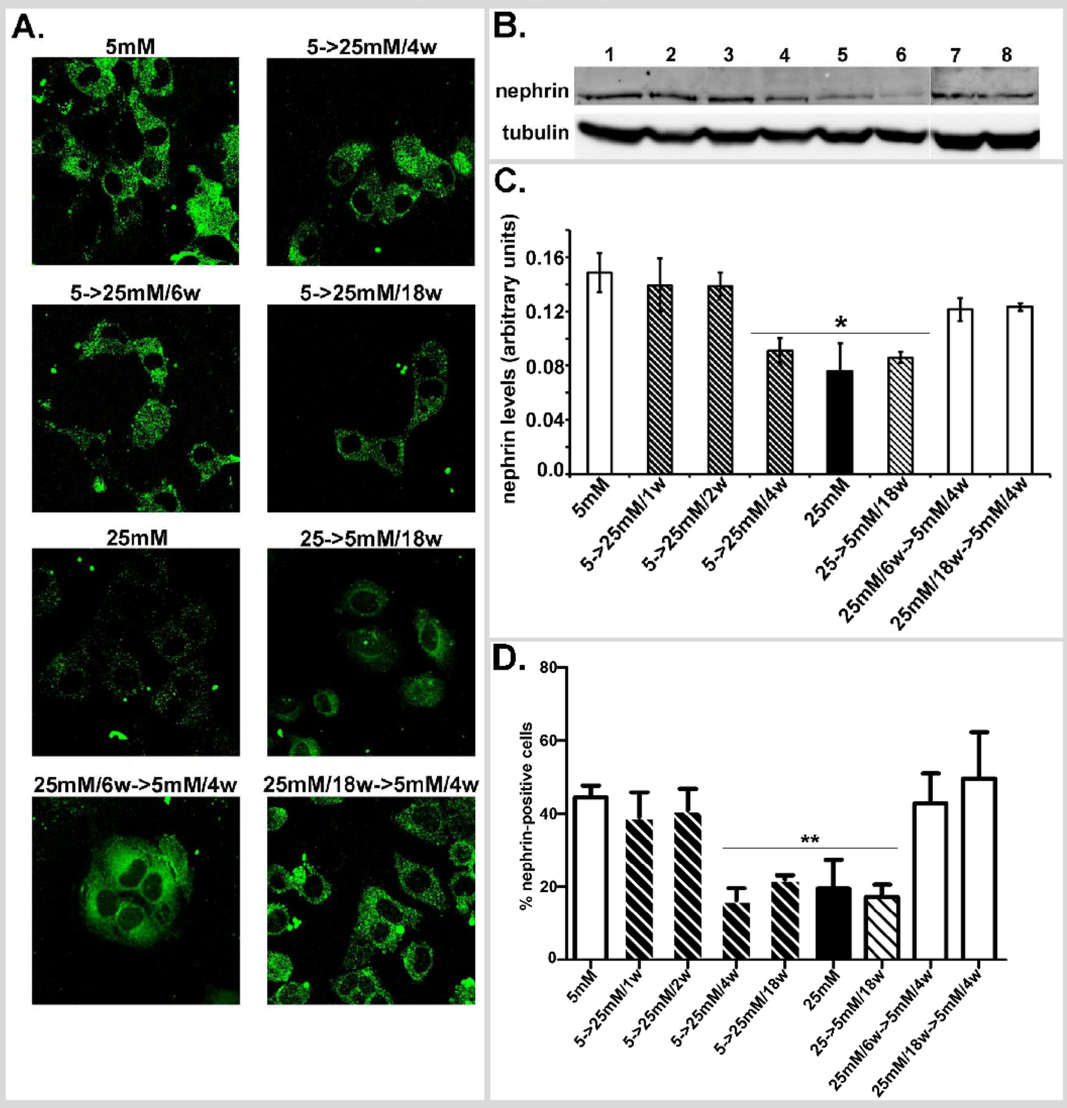

Figure 2: Nephrin cell surface expression in podocytes. (A) Confocal microscopic analysis (x600) for the distribution of nephrin in HGEC. Cells were fixed and stained with anti-nephrin antibody and AlexaFluor 488-conjugated anti-goat IgG as secondary antibody. (B) Western blot analysis for nephrin expression in HGEC. Total protein was extracted from cells cultured either in the presence of 5mM (control) (lane 1) or 25mM (lane 5) glucose. Lanes 2, 3 and 4 represent HGEC:5mM-to-25mM/1w, HGEC:5mM-to-25mM/2w and HGEC:5mM-to-25mM/4w respectively. Lane 6 represents HGEC:25mM-to-5mM/18w. Lanes 7 and 8 represent HGEC:25mM/6w-to-5mM/4w and HGEC:25mM/18w-to-5mM/4w respectively. Blots were reprobed with anti-tubulin antibody, to verify protein loads, against which the data were normalized. (C) Results were expressed as the mean ± SD of four independent experiments (*p<0.05 vs. HGEC:5mM). (D) FACS analysis of nephrin cell surface expression. Percentage of positive cells was calculated and presented for the histogram (**p<0.01 vs. HGEC:5mM).

❖ **The intracellular domain of nephrin associates with CD2AP, an adaptor molecule which plays a major role in the maintenance of podocyte phenotype due to its cytoskeleton stabilizing properties. In vitro culturing of HGEC in high glucose transiently reduced CD2AP expression.** (Figure 3).

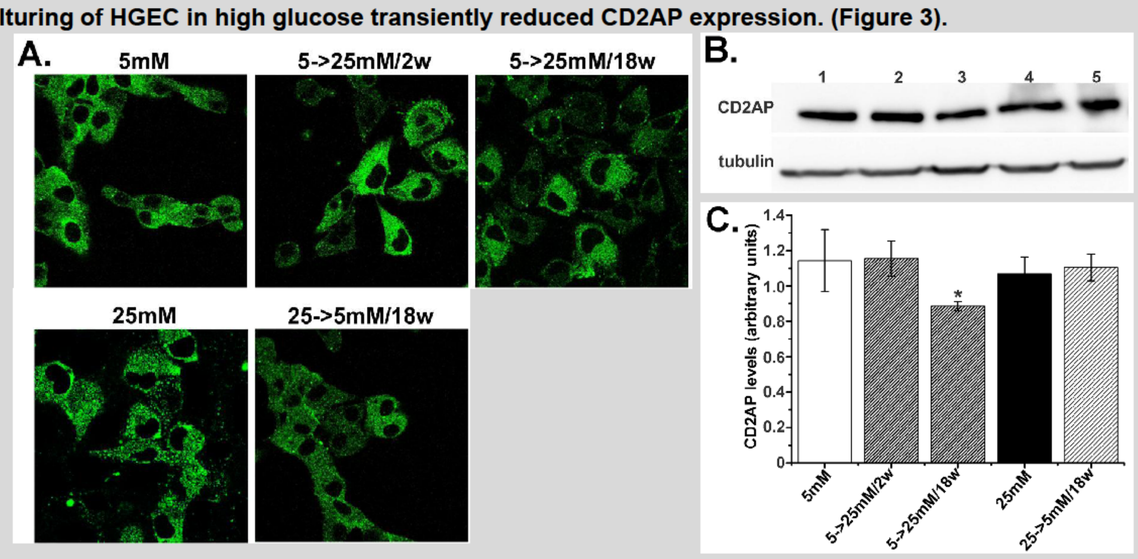

Figure 3: CD2AP expression in podocytes. (A) Confocal microscopic analysis (x600) for the distribution of CD2AP in HGEC. (B) Western blot analysis for CD2AP expression in HGEC. Total protein was extracted from HGEC:5mM (control) (lane 1) or HGEC:25mM (lane 4). Lanes 2 and 3 represent CD2AP expression in HGEC:5mM-to-25mM/2w and HGEC:5mM-to-25mM/18w respectively. Lane 5 represents CD2AP expression in HGEC:25mM-to-5mM/18w. Blots were reprobed with anti-tubulin antibody, (C) Results were expressed as the mean ± SD of three independent experiments (*p<0.05 vs. HGEC:5mM).

❖ **The antiadhesive protein podocalyxin (PC) is a pivotal podocytic marker which regulates podocyte morphology, as well as foot process formation and maintenance. In HGEC, glucose-induced reduction of PC expression started at 2 weeks of culture in 25mM glucose. Maximal downregulation was observed after 18 weeks of culture in 25mM glucose. Glucose-induced downregulation of PC expression is only partly reversible. (Figure 5).**

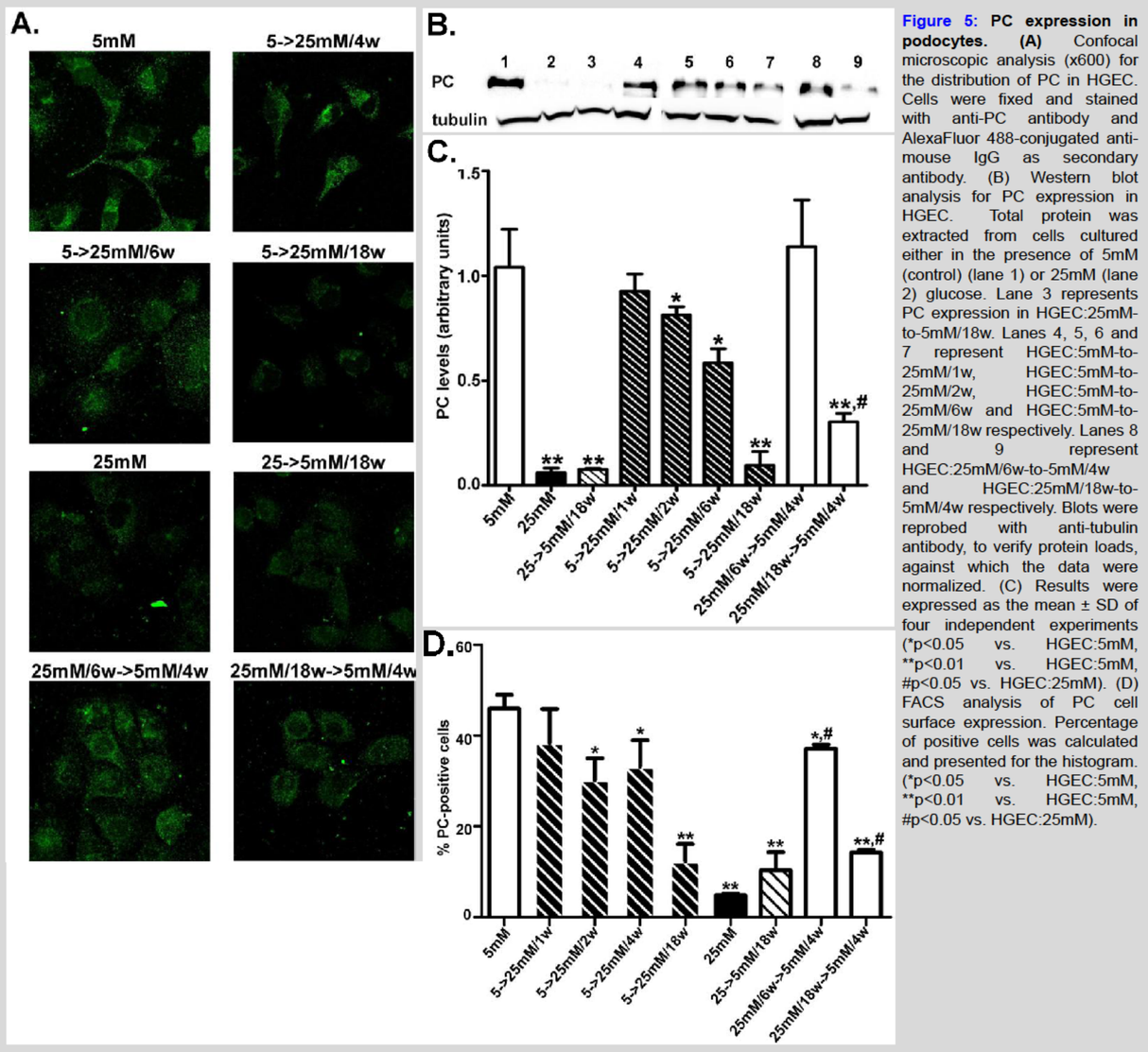

Figure 5: PC expression in podocytes. (A) Confocal microscopic analysis (x600) for the distribution of PC in HGEC. Cells were fixed and stained with anti-PC antibody and AlexaFluor 488-conjugated anti-mouse IgG as secondary antibody. (B) Western blot analysis for PC expression in HGEC. Total protein was extracted from cells cultured either in the presence of 5mM (control) (lane 1) or 25mM (lane 2) glucose. Lane 3 represents PC expression in HGEC:25mM-to-5mM/18w. Lanes 4, 5, 6 and 7 represent HGEC:5mM-to-25mM/1w, HGEC:5mM-to-25mM/2w, HGEC:5mM-to-25mM/6w and HGEC:5mM-to-25mM/18w respectively. Lanes 8 and 9 represent HGEC:25mM/6w-to-5mM/4w and HGEC:25mM/18w-to-5mM/4w respectively. Blots were reprobed with anti-tubulin antibody, to verify protein loads, against which the data were normalized. (C) Results were expressed as the mean ± SD of four independent experiments (*p<0.05 vs. HGEC:5mM, **p<0.01 vs. HGEC:5mM, #p<0.05 vs. HGEC:25mM). (D) FACS analysis of PC cell surface expression. Percentage of positive cells was calculated and presented for the histogram. (*p<0.05 vs. HGEC:5mM, **p<0.01 vs. HGEC:5mM, #p<0.05 vs. HGEC:25mM).

❖ **WT1 binding elements have been identified on the promoter of PC gene (*podxl*). In addition to the PC promoter, WT1 binds to the nephrin gene (*nphs1*) promoter and activates nephrin expression in podocytes WT1 binding in HGEC:25mM was decreased by >50% compared to HGEC:5mM. HGEC exposed to 25mM glucose for 6 weeks displayed a 2-fold reduction of WT1 binding. Even though PC expression at this early time point could be restored, binding of WT1 to *podxl* promoter remained decreased (Figure 6A). However, there were no significant differences in WT1 binding to the *nphs1* promoter in HGEC:5mM (expressing nephrin) or HGEC permanently exposed to high glucose (HGEC:25mM, not expressing nephrin). (Figure 6B)**

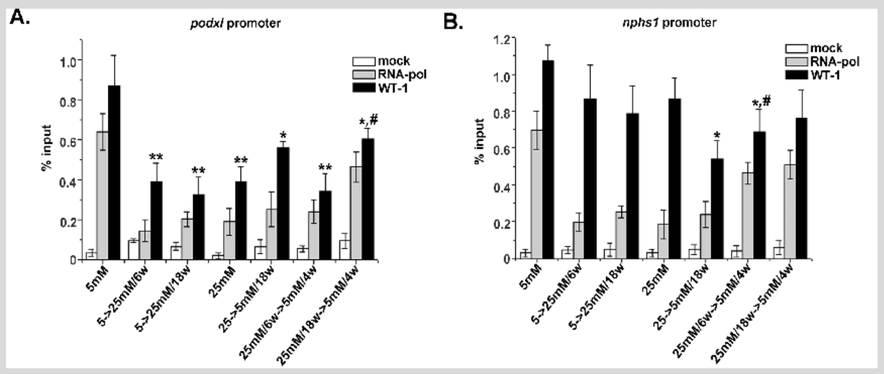

Figure 6: ChIP analysis of WT1 binding to *podxl* and *nphs1* promoter regions. Formaldehyde-crosslinked chromatin fragments were precipitated with either anti-WT1 antibody, anti-RNA polymerase II or no antibody as mock. Precipitated products were amplified by real-time PCR using primers for *podxl* or *nphs1* proximal promoter. As normalizer, a DNA fragment lacking any WT1 site, located in the promoter region of GAPDH gene, was used.

CONCLUSIONS

Our results indicate that:

- ❖ **The presence of high glucose induced a phenotypic conversion of podocytes resembling partial dedifferentiation.**
- ❖ **Dysregulation of the normal podocytic phenotype is an event differentially affecting the expression of function-specific podocytic markers.**
- ❖ **Downregulation of the epithelial marker CD10/CALLA and PC appeared first and was followed by stably downregulated nephrin.**
- ❖ **WT1 may not be directly involved with upregulation of previously reduced PC and nephrin expression.**
- ❖ **Investigation of factors that affect PC and nephrin expression is pivotal for maintenance of podocyte phenotype and survival.**

References

Reiser J, Kriz W, Kretzler M, Mundel P: The glomerular slit diaphragm is a modified adherens junction. J Am Soc Nephrol 2000, 11(1):1-8.

Li Y, Kang YS, Dai C, Kiss LP, Wen X, Liu Y: Epithelial-to-mesenchymal transition is a potential pathway leading to podocyte dysfunction and proteinuria. Am J Path 2008, 172(2):299-308.

Drossopoulou GI, Tsotakos NE, Tsilibary EC: Impaired transcription factor interplay in addition to advanced glycation end products suppress podocalyxin expression in high glucose-treated human podocytes. Am J Physiol Renal Physiol 2009, 297(3):F594-603.

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Diabetes - experimental models

Garyfalia Drossopoulou

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