EXPLORING ALBUMIN UPTAKE IN HUMAN PODOCYTES: A POSSIBLE INVOLVEMENT FOR THE TUBULAR UPTAKE MACHINERY

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INTRODUCTION AND AIMS

Loss of proteins is the hallmark of tubular and glomerular diseases, and may be due to structural and/or functional alterations involving different cell types [1]. Albuminuria is a powerful independent predictor of renal disease progression, cardiovascular disease, and death in patients with renal disease, hypertension, diabetes, and vascular disease, and in the general population too [1]. Protein endocytosis at tubular level relies on an active receptor-mediated pathway that mainly involves megalin (*LRP2*), cubilin (*CUBN*), amnionless (*AMN*), disabled-2 (*DAB2*) and ClC-5 (*CLCN5*) [1]. Cubilin forms a functional complex with AMN named CUBAM, which is translocated to the plasma membrane and exhibits megalin-independent activity [2,3]. Like renal PTECs, podocytes have been found capable of internalizing albumin. Several Authors described and quantified an albumin endocytosis function in murine and rat podocytes, both *in vitro* and *in vivo* [4,5]. Recently, it has been demonstrated that human podocytes can endocytose proteins such as albumin using kinetics consistent with a receptor-mediated process when placed in a medium with high doses of albumin [6,7]. In the last few years, human podocytes have been shown to express megalin and cubilin, both *in vivo* and *in vitro* [8,9]. Our own group found ClC-5 expressed in the glomeruli of normal and proteinuric human kidneys, and particularly in podocytes [10]. In the same study, ClC-5 was overexpressed at both mRNA and protein level in the glomeruli of biopsies obtained from patients with diabetic nephropathy and membranous glomerulonephritis.

How human podocytes perform endocytosis is still unclear. The aim of the present study was therefore to clarify whether components of the typical tubular protein uptake system - such as megalin, cubilin, ClC-5, AMN and Dab2 - are involved in the mechanism underlying albumin internalization in human podocytes *in vitro*, and how protein overload might affect the expression of this system.

METHODS

To confirm the presence of an uptake mechanism, we performed overnight time lapse experiments with a low dose of FITC-BSA (10 µg/ml).

To evaluate the uptake kinetic, we stimulated podocytes with FITC-BSA at different time (2, 4, 8 h) and doses (10 µg/ml, 100 µg/ml and 1 mg/ml) both at 37 and 4°C.

Immunofluorescence was performed to evaluate the presence of ClC-5, megalin, cubilin, Dab2 and amnionless in human podocytes cultivated in standard condition and stimulated with FITC-BSA (10 µg/ml) at different time points (2, 4, 8 and 24 h).

Finally, we stimulated human podocytes with increasing concentrations of BSA (range 10 µg/ml - 30 mg/ml), evaluating changes in gene and protein expression of the components of this system by Real Time PCR or In Cell Western respectively at different time points (24, 48 and 72 h).

To see whether cubilin mediates albumin uptake, a sheep polyclonal antibody against the extracellular domain of the receptor (6 µg/ml) was added to podocyte media 3 hours before stimulation.

RESULTS

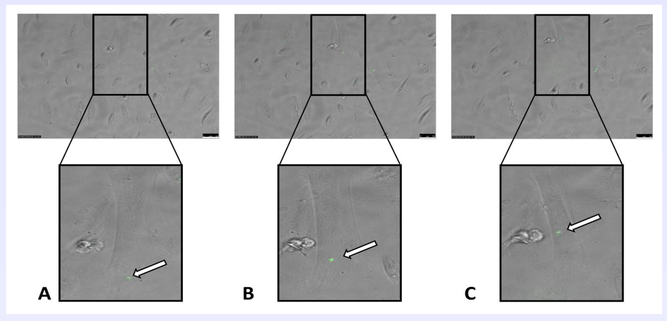

Figure 1. Podocytes perform albumin uptake even at low doses. Time lapse experiments disclosed the ability of podocytes to internalize albumin (FITC-BSA 10µg/ml). Representative images show vesicles containing albumin (green) at different time points: 5 h 30 min (A), 6 h 39 min (B), 8 h 18 min (C). The white arrow follows the movement of one vesicle from the periphery of the cell to the perinuclear region. Objective 20X/0.4. Scale bar 50 µm.

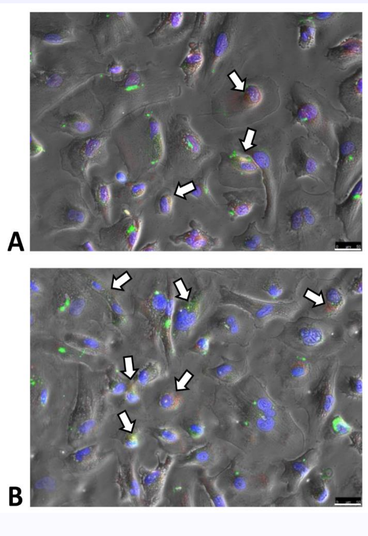

Figure 4. Cubilin significantly colocalizes with albumin. Immunolabeling shows colocalization (in yellow/orange) of megalin (A) and cubilin (B) with albumin after 24 hours of incubation. Statistical analysis using Pearson's coefficient (*Rr*) with LAS-AF software disclosed a significant correlation between cubilin and FITC-BSA (*Rr*=0.71), and a non-significant correlation between megalin and FITC-BSA (*Rr*=0.45). Green: FITC-BSA; Red: megalin (A) or cubilin (B); Blue: DAPI. White arrows indicate colocalization. Objective 20X/0.4. Scale bar 50 µm.

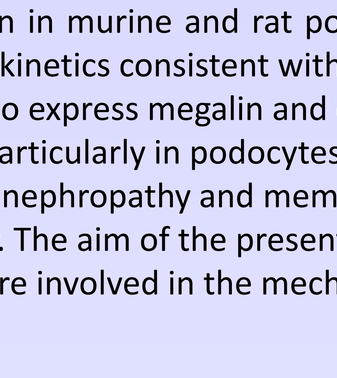

Figure 2. Albumin uptake follows receptor-mediated kinetics in human podocytes. After 2 hours of stimulation, FITC-BSA was significantly internalized at all doses tested by comparison with non-stimulated control cells. Albumin uptake increased until saturation at 37°C (continuous line), with abolition of the process at 4°C (dotted line) (A). Graph B shows significant quantity of FITC-BSA internalized by podocytes at 37°C comparing to 4°C. Data are shown as the average ± SD of three different experiments in triplicate * p<0.05; § p<0.01.111

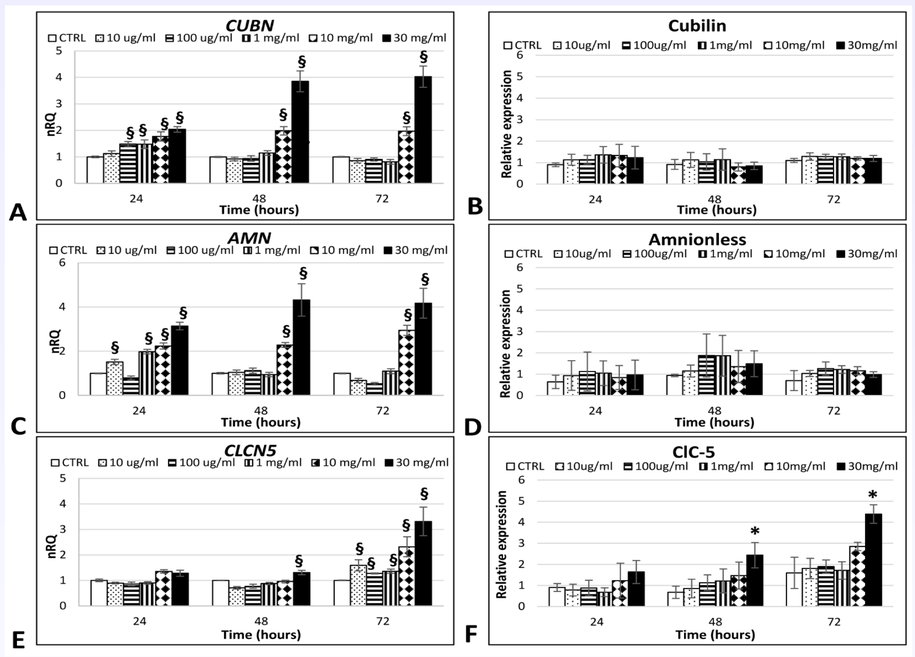

Figure 5. Protein overload modulates the expression of the CUBAM complex. Gene expression analysis of the macromolecular system showed an increase in *CUBN* (A), *AMN* (C) and *CLCN5* (E) genes. Data are given as the geometrical average ± SE of three independent experiments in duplicate. Protein expression analysis of the macromolecular system only showed an increase in ClC-5 expression (F) at the longest time and highest dose considered. Data are given as the average ± SD of four different experiments. nRQ: normalized Relative Quantity, CTRL: control cells, *p<0.05, §p<0.01.

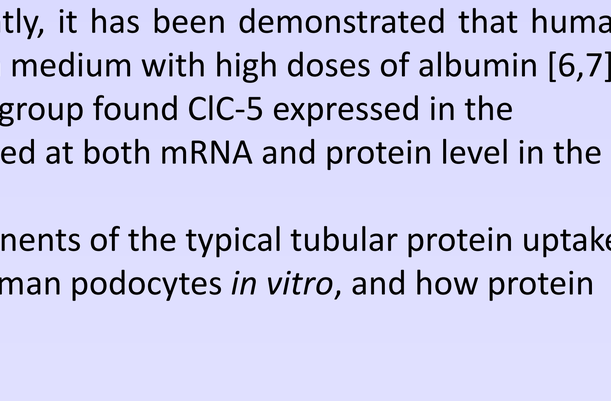

Figure 3. Podocytes express the typical tubular uptake machinery. Immunostaining of human podocytes disclosing: A podocin (red), B ClC-5 (brown), C megalin (red), D cubilin (green), E Dab2 (red), F AMN (red). Blue: DAPI. Objective 20X. Scale bar 50 µm.

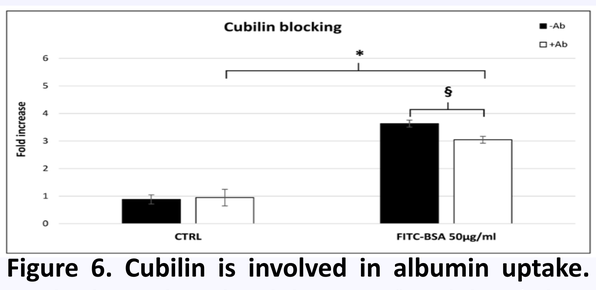

Figure 6. Cubilin is involved in albumin uptake. Antibody-mediated inhibition of cubilin (white blocks) led to a significant decrease in albumin endocytosis, indicating an involvement of this receptor in the protein uptake process in mature podocytes. Data are shown as the average ± SD of two different experiments in triplicate. CTRL: control cells, * p<0.05, § p<0.01.

CONCLUSIONS

For the first time, we demonstrated that human podocytes are committed to performing endocytosis *via* a receptor-mediated mechanism even in the presence of low doses of albumin, thus supporting their ability to do so. We identified the components of the typical tubular protein uptake system - such as megalin, cubilin, ClC-5, AMN and Dab2 - in human podocytes *in vitro* too. We demonstrated that protein overload first acts on the expression of the CUBAM complex in these cells, then involves the ClC-5 channel in the endocytic vesicle acidification. Although human podocytes express the components of the tubular uptake machinery, they seem to perform the receptor-mediated endocytosis of albumin in different ways from PTECs. In fact, albumin uptake in PTECs requires coordinated collaboration between megalin and cubilin, while cubilin was found to have a more important role than megalin in human podocytes. Our findings provide the first clear evidence of the CUBAM complex having an active role in albumin endocytosis in human podocytes *in vitro*.

REFERENCES

[1] Gianesello, L., et al. J. Clin. Nephrol. Kidney Dis. 1, 1002 (2016).
[2] Fyfe, J.C., et al. Blood. 103, 1573-1579 (2004).
[3] Coudroy, G., et al. J. Am. Soc. Nephrol. 16, 2330-2337 (2005).
[4] Eyre, J., et al. Am. J. Physiol. Renal Physiol. 292, F674-F681 (2007).
[5] Kinugasa, S., et al. Kidney Int. 80, 1328-1338 (2011).
[6] Dobrinskikh, E., et al. Am. J. Physiol. Renal Physiol. 306, F941-F951 (2014).
[7] Pawluczyk, I.Z., et al. Exp. Cell Res. 326, 251-258 (2014).
[8] Prabakaran, T., et al. PLoS One. 6, e25065 (2011).
[9] Prabakaran, T., et al. Nephrol. Dial. Transplant. 27, 3156-3159 (2012).
[10] Ceol, M., et al. PLoS One. 7, e45605 (2012).

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