ClC-5 AND PROTEINURIC NEPHROPATHIES

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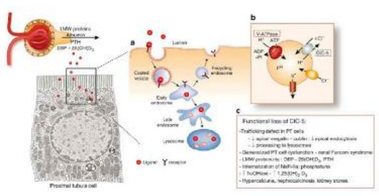
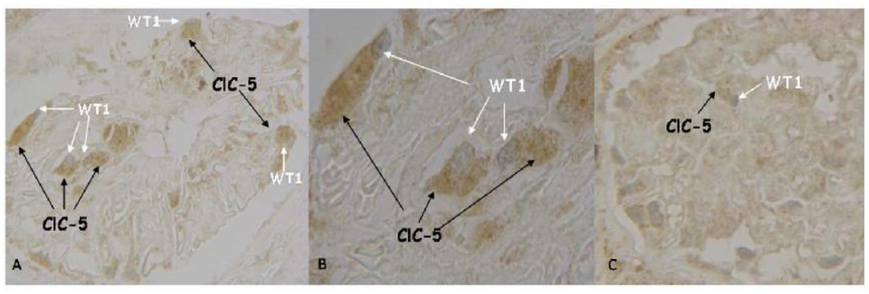
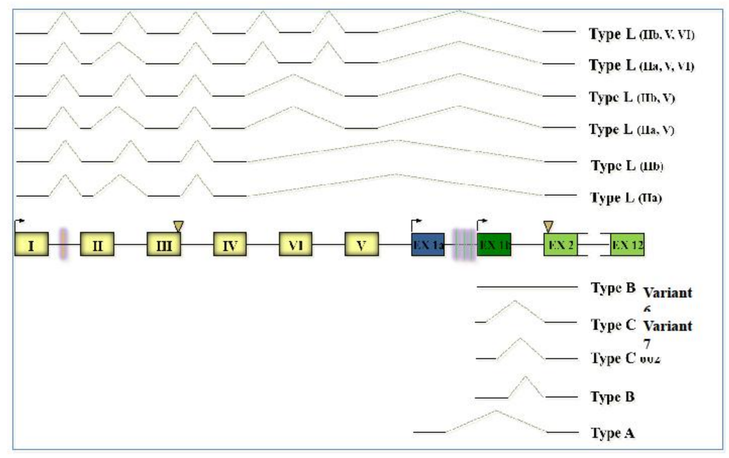
BACKGROUND

CLC-5, the electrogenic chloride/ proton exchanger, is a member of the CLC family of voltage-gated chloride channels and transporters. In the kidney ClC-5 is expressed in the S3 segment of proximal tubules, in the ascending limb of Henle's loop, and in the intercalated cells of the collecting duct (1). Recently we demonstrated its expression in podocytes of same proteinuric nephropathies (2). ClC-5 is crucial for the renal reabsorption of low molecular weight proteins that can pass the glomerular filter into the primary urine. It provides an electric shunt for the vesicular H+-ATPase which is needed for an efficient acidification of endosomes.





The human *CLCN5* gene, spanning about 170 kb of genomic DNA on chromosome Xp11.23/p11.22, consists of 17 exons including 11 coding exons (2-12). The presence of many different 5'UTR ends of *CLCN5* mRNA in the kidney highlights the complexity of both the molecular structure and the regulatory apparatus of the gene. As a results of alternative splicing in some 5' UTR exons, 11 different mRNAs are generated in human kidney. ORF analysis predicts that all isoforms except three variants encoded for the canonical 746 aa ClC-5 protein (3). The functional significance of these regulatory regions has not been elucidated. It has been reported that different 5'UTR ends present in various tissues may serve to differently regulate gene expression in response to physiological and pathological stimuli through mechanisms involving not only transcription but also translation



AIM

To go deepen the role of ClC-5 on proteinuric nephropathies, we investigated mRNA expression of *CLCN5* gene and its different 5'UTR isoforms as well as ClC-5 protein in renal biopsies of patients with different degree of proteinuria.

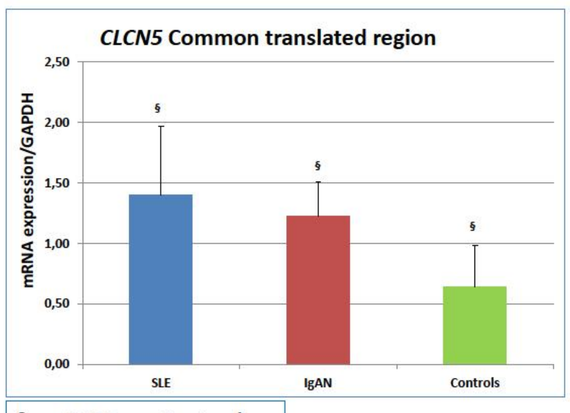
MATERIAL AND METHODS

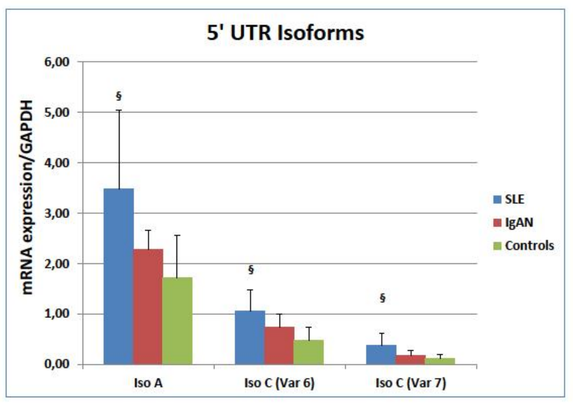
Total RNA was isolated with silica gel columns (RNeasy mini Qiagen) from 12 LES (3,31 ± 2,5 g/die), 14 IgA Nephropathies (IgAN) 2,35 ± 1,06g/die, and 11 Controls (C) from the tumor-bearing renal tissue and was reversed transcripted with Random Examers. *CLCN5* translated region, 5'UTR isoform A and isoforms C (variant 6 and 7) were analyzed by quantitative comparative RT-PCR using *GAPDH* (450bp) as housekeeping gene.
Indirect immunohistochemistry with primary rabbit anti-human ClC-5 antibodies (Sigma) was used and ClC-5 signal was quantified by morphometric analysis (using Image Pro Plus software, Media Cybernetics) at 200X magnification in glomerular and tubular compartments.
Student's t-test and regression analysis were performed. A p-value ≤ 0,05 was considered statistically significant.

RESULTS

mRNA expression study

The relative mRNA expression of *CLCN5* translated region was significantly higher in MG than in C and DN, and significantly higher in LES than in C. Isoform A and C were also significantly higher in MG and in LES than in C. Isoform A was more abundant than Isoform C. Moreover, a significant direct correlation was found between Isoform A and Isoform C (417 variant) (r=0,64 p=0,000).



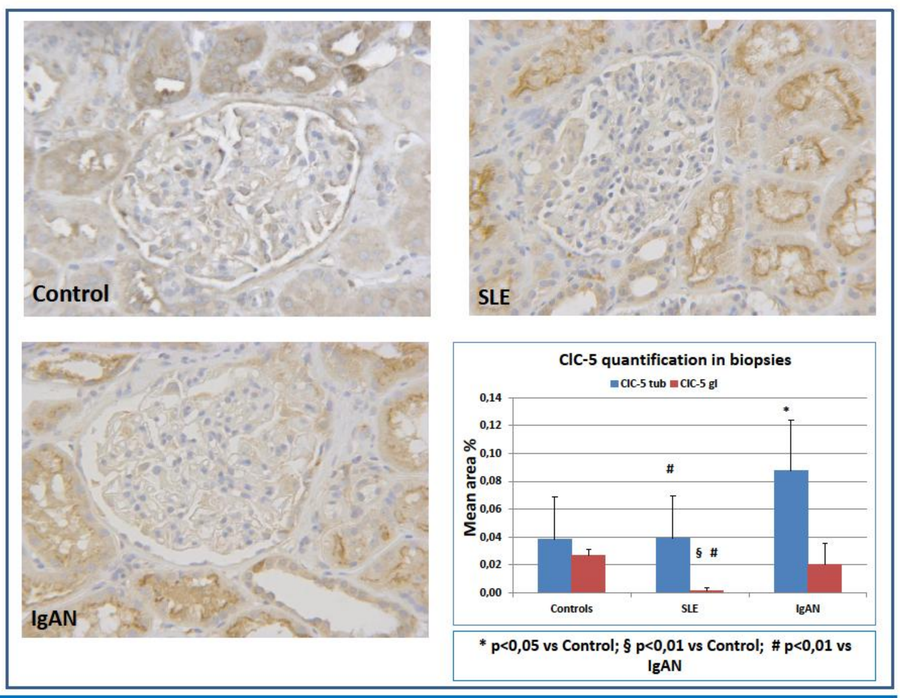


§ p<0,01 vs Control

Protein expression study

The study at protein level was focused on glomerular and tubular compartments and revealed that :

1) At tubular level ClC-5 was significantly higher in IgAN vs Controls and SLE (p=0,04 and p=0,007 respectively); at glomerular level ClC-5 was significantly higher in IgA versus SLE (p=0,002) and in Controls versus SLE (p=0,000);
2) Tubular and glomerular ClC-5 protein expression was directly correlated considering all the nephropathies (r=0,76 p=0,016);
3) Proteinuria did not correlate with ClC-5 protein expression except in SLE with nephrotic syndrome where it correlated with glomerular expression (r=0,68 p=0,009).



* p<0,05 vs Control; § p<0,01 vs Control; # p<0,01 vs IgAN

CONCLUSIONS

Our study clearly confirms that ClC-5 protein is expressed in glomeruli, but also reveals that ClC-5 expression is differently modulated at mRNA and protein level, being proteinuria probably a secondary actor of ClC-5 regulation.
Moreover, immunosuppressive therapy seams to downregulate the expression of ClC-5 both in tubular and glomerular compartment.

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