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Large T antigen is critical for Transglutaminase 2 extracellular trafficking and is associated with renal function

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Background

Tissue transglutaminase (TG2) cross-links extracellular proteins that resulted in the accumulation of extracellular matrix (ECM) proteins. The accumulation of ECM protein is the initial event for kidney fibrosis. To cross-link ECM proteins, TG2 must be transported into extracellular space in the process of fibrosis. TG2 has no signal peptide and cannot be transported classically. It is unknown how TG2 is targeted to the cell surface and secreted into ECM. Understanding of the extracellular trafficking process may help to development novel treatment of kidney fibrosis. We identified that amino acids 88-106 in N-terminal β-sandwich domain[1] of TG2 molecule is crucial for TG2 externalisation using deletion and mutation analysis in three renal tubular epithelial cells. We explored possible transport patterns of TG2, the protein-protein interaction was confirmed using co-immunoprecipitation, and identified protein in TG2 extracellular trafficking was further explored using human tissue sample.

Methods

Protein interaction was screened using yeast two hybrid technique and the TG2 extracellular trafficking experiments using NRK52E cell line. Yeast two hybrid (Y2H) screening was performed using Matchmaker Gold Yeast Two-hybrid System (Cat. No. 630489, Clontech, UK) and the TG2 export motif aa88-106 was used as bait. The kidney tissue samples were obtained from the tissue bank of China Medical University Hospital.

Results

The TG2 export motif aa88-106 was cloned into bait vector pGBKT7 and two-hybrid library screening with yeast mating using a normalized human protein library was performed. The DNA sequences were identified using BLAST. We identified large T antigen (LTA) is one of the identified binding partners of TG2 using yeast two hybrid technique and anti-sense treatment of LTA may decrease TG2 extracellular trafficking in tubular epithelial cells. The protein-protein interactions was supported by the findings of co-immunoprecipitation (Figure 1).

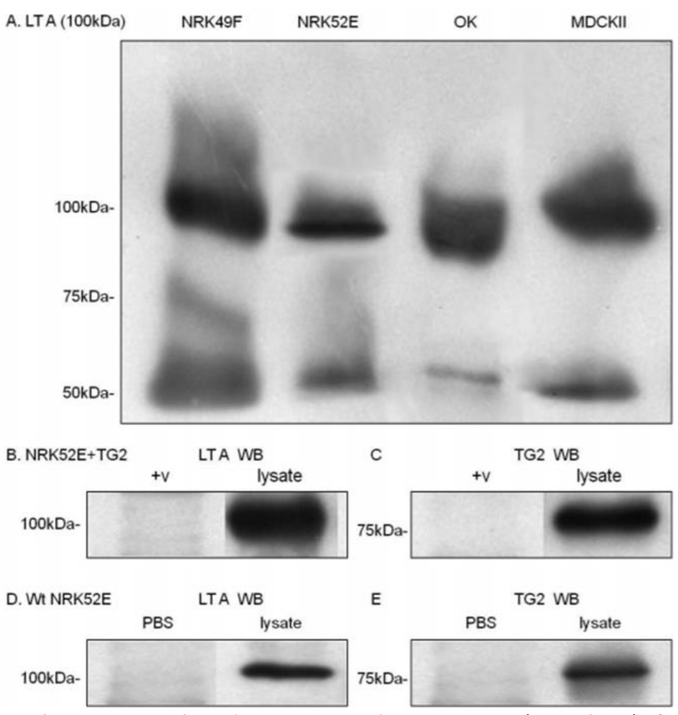

Figure 1 Co-immunoprecipitation of large tumour antigen (LTA) and TG2
A Western blot (WB) immunoprobed with an anti-LTA antibody using cell lysate from wild type NRK52E, NRK49F, OK and MDCKII cells confirmed the expression of LTA (A). A WB of cell lysate from NRK52E cells transfected with pcDNA6.2/cTC-Tag-dest vector containing full length TG2 cDNA immunoprecipitated using an anti-TG2 antibody (cub7402) was immunoprobed for LTA giving a band at 100 kDa that corresponds to LTA (B). Immunoprobing a WB for TG2 from a LTA pulldown showed a band at 75 kDa that corresponds to TG2 (C). LTA-TG2 interaction at endogenous protein levels was determined using cell lysates from wild type NRK52E. This gave a band corresponding to LTA (100 kDa) from a TG2 pulldown (D) and a band corresponding to TG2 at 75 kDa (E) from a LTA pulldown suggesting a protein-protein interaction at endogenous protein levels in NRK52E cells.

LTA cane be identified in cells lines including OK, NRK52E, MDCK II. The anti-sense treatment of LTA resulted in a 50% decrease of LTA in these cells and this also decreases the extracellular TG2 . (Figure 2)

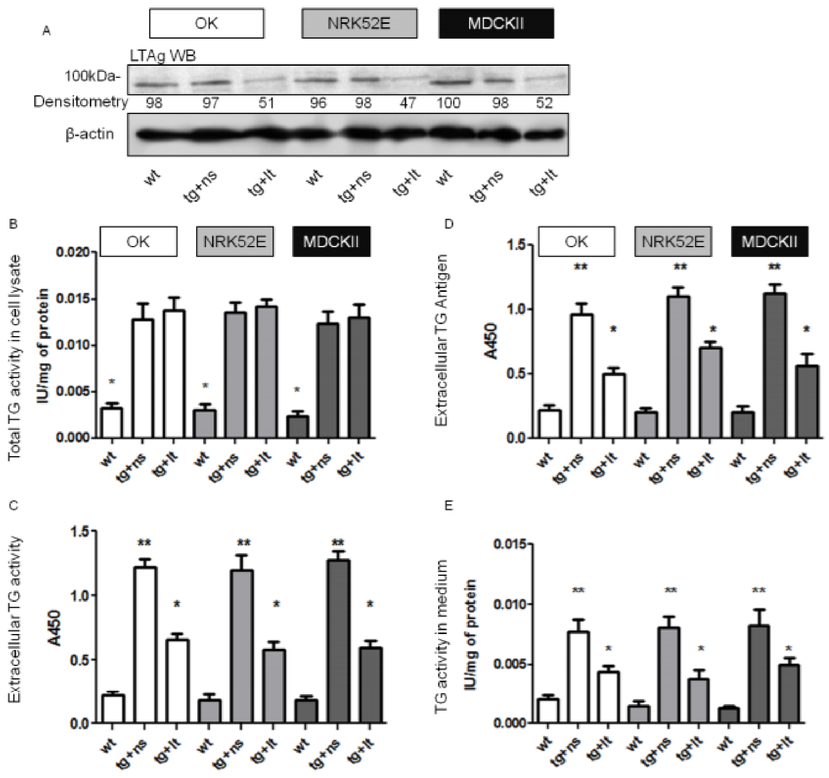

Figure 2 Extracellular and intracellular TG activity following large T-antigen (LTA) knockdown
OK, NRK52E and MDCKII cells were co-transfected with pcDNA6.2/cTC-tag-DEST expression vector containing TG2 cDNA (tg) with either non-sense siRNA (tg+ns) or anti-LTA siRNA (tg+lt). Non-transfected cells (wt) were used as a negative control. Western blots from cell lysates were immunoprobed for LTA (A) and densitometry used to quantify LTA expression. The intracellular TG activity was determined by a peptide incorporation assay (B). The extracellular TG activity (C), extracellular TG antigen (D) and TG activity in medium (E) were assayed as previously described. Data represents: Mean ± SEM, n=5, *p<0.05, **p<0.01.

However, the finding may be interfered in the cell line experiments because LTA may be introduced in the transformation process. LTA can be produced by polyoma virus such as BK virus (BKV) in human. We accessed the expression of LTA in kidney tissue samples from patients with and without BK virus infection. Membrane LTA expression[2] is found in the tissue samples from BKV(+) patients (n=10) but not in BKV(-) patients (n=10). The ECM TG2, collagen IV are significantly higher and estimated glomerular filtration rate (eGFR) is lower in BKV(+) patients (Figure 3).

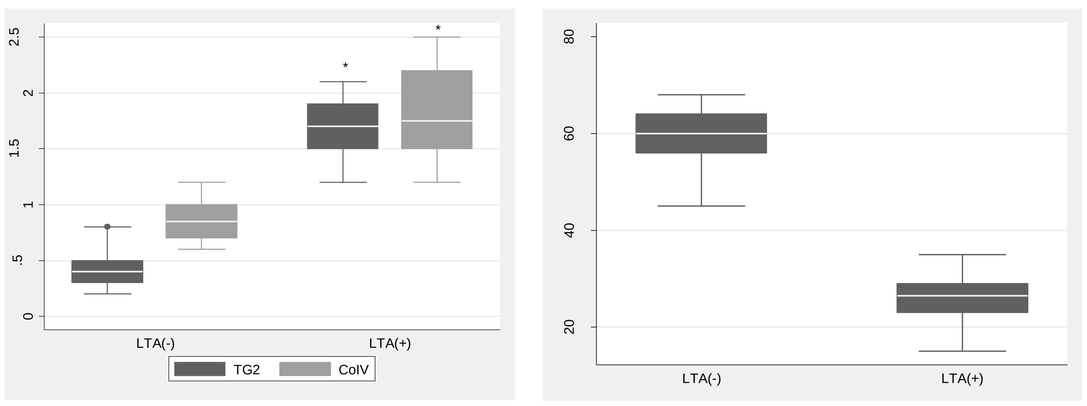

Figure 3 TG2, Collagen IV, and eGFR in patients with and without LTA expression in the cell membrane
Cell membrane LTA was only identified in patients with BKV infection (n=10) but not in the patients without (n =10). The TG and Collagen IV were significant higher in the extraceullar matrix component of the kidney tissue samples. The levels of TG2 and Collagen IV were corrected for the total protein concentration. The patients with BKV infection and the age-, gender-, matched patients without BKV infection were randomly selected from the tissue bank. The patients with LTA expression had lower estimated glomerular filtration rate (eGFR) than those without LTA expression on the cell membrane component.

Conclusions

Cell membrane LTA facilitate TG2 extracellular trafficking, led to kidney fibrosis, and decrease the eGFR. This may provide a potential novel treatment for kidney fibrosis i.e BK virus vaccine prevent kidney tissue from expression LTA, decrease TG2 extracellular trafficking and thus decrease kidney fibrosis.

References

1. 1. Chou, C.Y., et al., *A crucial sequence for transglutaminase type 2 extracellular trafficking in renal tubular epithelial cells lies in its N-terminal beta-sandwich domain.* J Biol Chem, 2011. **286**(31): p. 27825-35.
2. Naba, A., K.R. Clauser, and R.O. Hynes, *Enrichment of Extracellular Matrix Proteins from Tissues and Digestion into Peptides for Mass Spectrometry Analysis.* J Vis Exp, 2015(101): p. e53057.