PROGRESSION OF TUBULAR DAMAGE IN DIABETIC NEPHROPATHY IS LINKED TO AN ALTERED UBIQUITINATION OF THE CYTOSKELETON

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BACKGROUND

- ✓ Chronic hyperglycemia, causes renal lesions in 20-30% of patients leading to diabetic nephropathy (DN), the principal cause of end-stage renal disease.
- ✓ Tubular cells play an important role in the pathogenesis of DN, but the molecular damage induced by hyperglycemia is almost unclear.
- ✓ Among the pathways known to be involved in the onset of DN, the ubiquitin pathway is emerging as a possible key-player. UBE2V1, an ubiquitin-conjugating E2 enzyme variant, mediates the formation of lysine 63 (lys63)-linked ubiquitin chains, affecting protein localization and cell signaling.

AIM

Aim of our study was to evaluate the role of protein ubiquitination in response to hyperglycemia and its possible involvement in the progression of tubular damage in DN.

RESULTS

- ❑ UBE2V1 gene expression was increased in HK2 cells after HG stimulation (Figure 1)
- ❑ UBE2V1 protein expression and Lys63-ubiquitinated proteins (K63) were increased on HK2 cells under HG conditions *in vitro* (Figure 2), and *in vivo* on FFPE tissues biopsies of patients with DN (Figure 3).
- ❑ The identification of lys63-linked ubiquitinated proteins by MALDI-TOF/MS-MS led to the discovery of 28 proteins specifically ubiquitinated in Lys63 under HG conditions, mainly involved in cellular assembly and organization (Figure 4)
- ❑ Chronic HG exposure lead to a subsequent depolymerization of actin cytoskeleton and the loss of vimentin (Figure 5).
- ❑ UBE2V1 silencing in HK2 cells under HG conditions reported to basal condition the actin ubiquitination in Lys63 (Figure 6).

CONCLUSIONS

- ➢ UBE2V1 and Lys63-poly-ubiquitinated proteins increase under HG conditions and persist in DN patients, mainly affecting cytoskeleton organization. This event could compromise cell survival and function, leading to progression of tubular damage during DN.
- ➢ Cytoskeletal ubiquitination could represent a potential therapeutic target to reduce tubular damage in the progression of DN.

Gene and protein expression of UBE2V1 and Lys63-ubiquitinated (k63) proteins in vitro and in vivo in DN patients

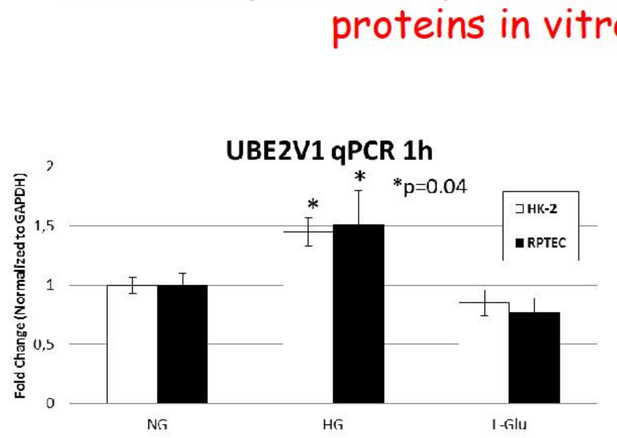

Figure 1: UBE2V1 gene expression under high glucose conditions in HK2 cells

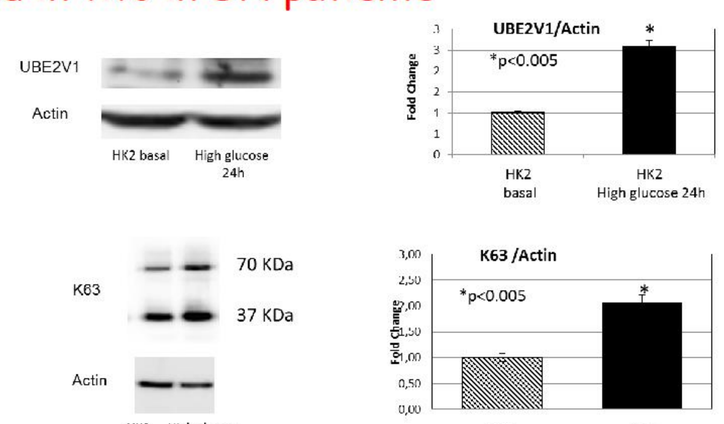

Figure 2: UBE2V1 and K63 protein expression under high glucose conditions in HK2 cells

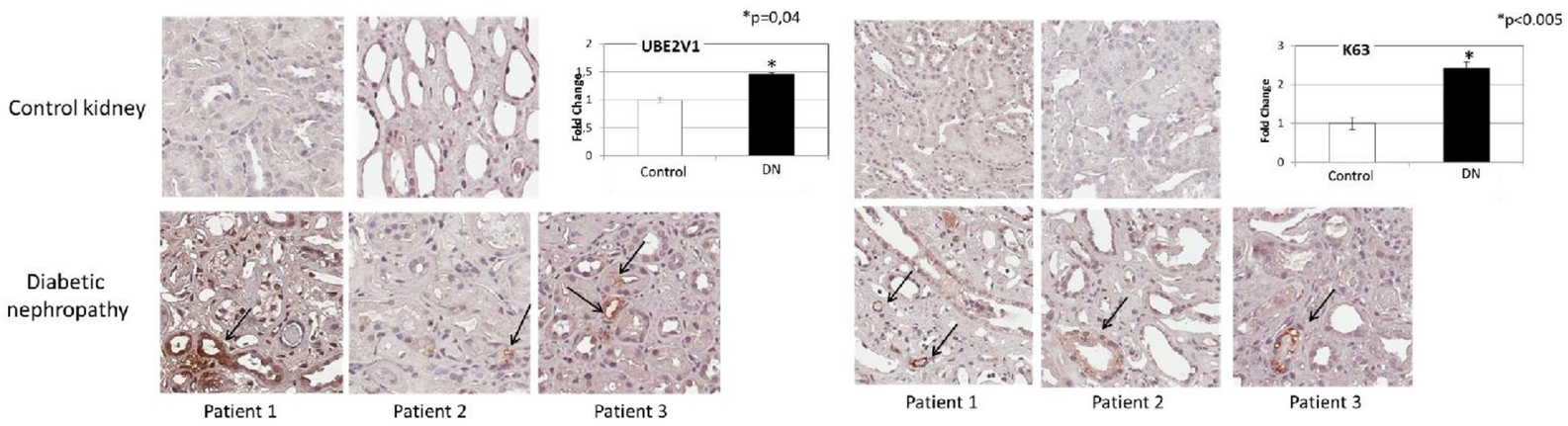

Figure 3: UBE2V1 and K63 protein expression *in vivo* in DN patients

HK2 cells under HG conditions shows specific actin lysin63 ubiquitination and depolymerization of actin cytoskeleton dependent by UBE2V1 activation

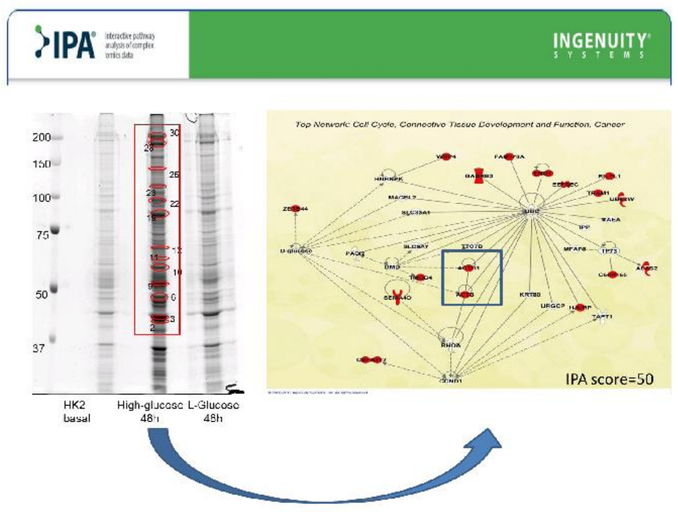

Figure 4: Identification by MALDI-TOF/MSMS of K63 proteins under high glucose condition in HK2 cells. IPA analysis of the functional networks.

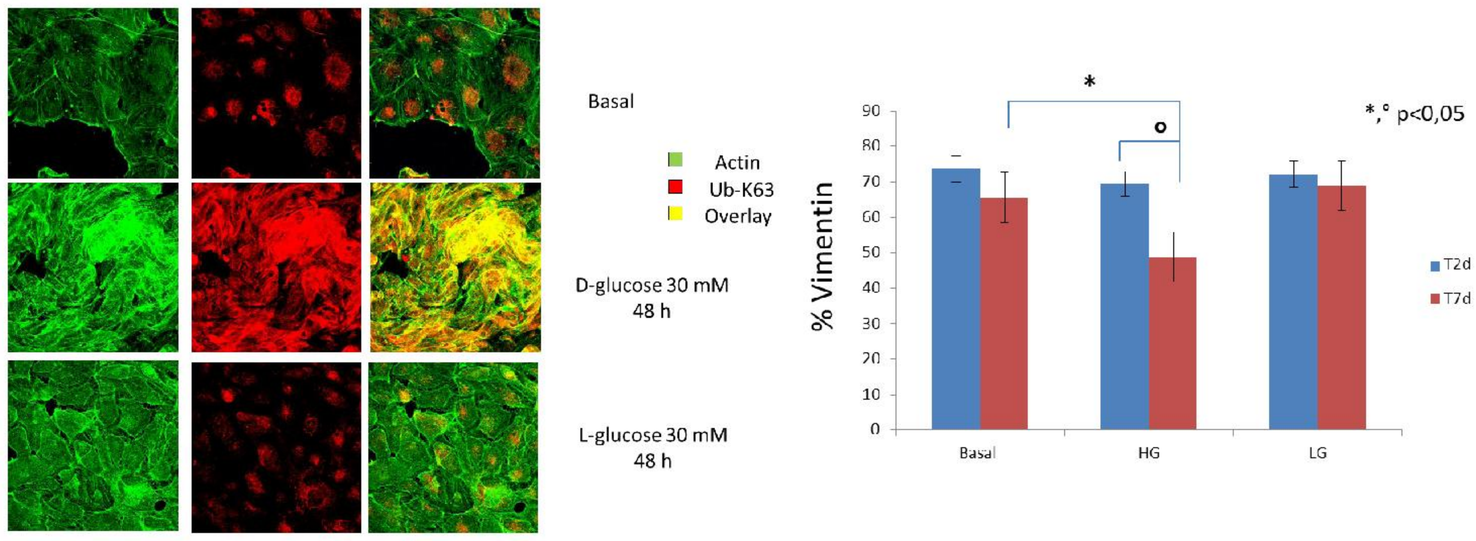

Figure 5: High glucose condition in HK2 cells lead to actin depolimerization and loss of vimentin

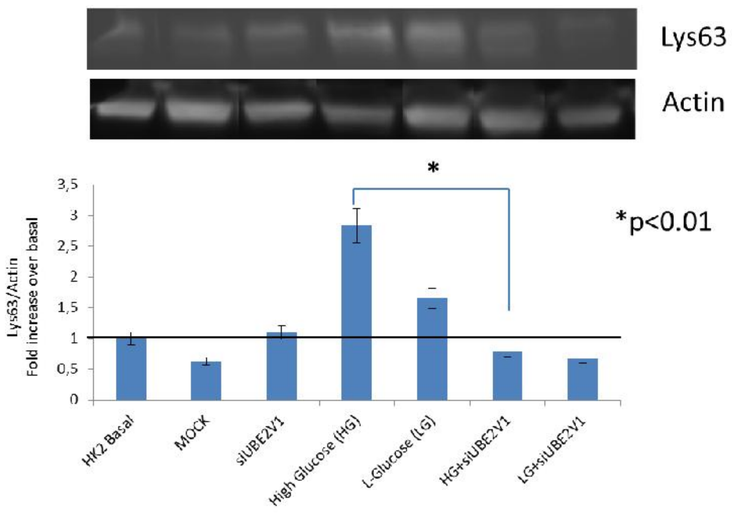

Figure 6: Effect of UBE2V1 silencing on K63 actin- ubiquitination in HK2 cells.

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I) Diabetes. I1) Basic research in diabetes.

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