Chronic Hyperglycemia Activate Autophagy Through An Increased K63 Linked Ubiquitination: A Candidate Pathogenic Mechanism In The Progression Of Tubular Damage In Diabetic Nephropathy

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BACKGROUND

- ✓ Chronic hyperglycemia, a key pathogenic factor of diabetic nephropathy (DN), can alter the autophagic cellular machinery, leading to progression of renal damage (1).
- ✓ The role of authophagy in proximal tubular cells under hyperglycemic conditions remain still controversial.
- ✓ We previously described that lysine63-ubiquitination plays a key role in the progression of tubular damage in DN (2); moreover it has been demonstrated that lysine 63-ubiquitination promotes the protein autophagic clearance (3).

AIMs

1. To evaluate *in vivo* the state of autophagy in diabetic patients without renal damage and in different classes of DN patients (classified in accordance to 4);
2. To investigate the role of Lysine 63 ubiquitination in the modulation of the autophagic processes in tubular cells (HK2 cells).

RESULTS

- ❑ Immunohistochemistry on kidney biopsies revealed an increased expression of LC3 authophagic factor at tubular level already in diabetic patients when compared to control biopsies **(Figure 1)**; autophagy persisted in all DN classes and in class IV patients we observed the activation of autophagic induced cell-death at tubular level **(Figure 1)**.
- ❑ Interestingly, the same tubules involved in the activation of autophagy (identified by LC3 staining), showed the presence of lysine 63 ubiquitinated proteins both in diabetic and in DN patients **(Figure 2)**.
- ❑ In order to clarify the role of lysine 63 ubiquitination in the regulation of autophagy, HK2 cells were grown under hyperglycemic conditions (HG: 30mM). Silencing of UBE2v1, an E2 enzyme involved in lysine 63 linked ubiquitination, completely abolished LC3 induced protein expression under HG conditions after 24h of stimulation **(Figure 3)**.
- ❑ Moreover, confocal microscopy revealed the disappear of autophagic vesicles induced by HG , in the presence of the specific inhibitor of lys63 ubiquitination (NSC697923) **(Figure 4)**.

CONCLUSIONS

- ➢ In conclusion, our data demonstrate that chronic hyperglycemia induce an increase in autophagy, linked to the accumulation of K63 ubiquitinated proteins. However, excessive or uncontrolled levels of autophagy could lead to autophagy-dependent tubular cell death, thus leading to the progression of renal damage in DN patients

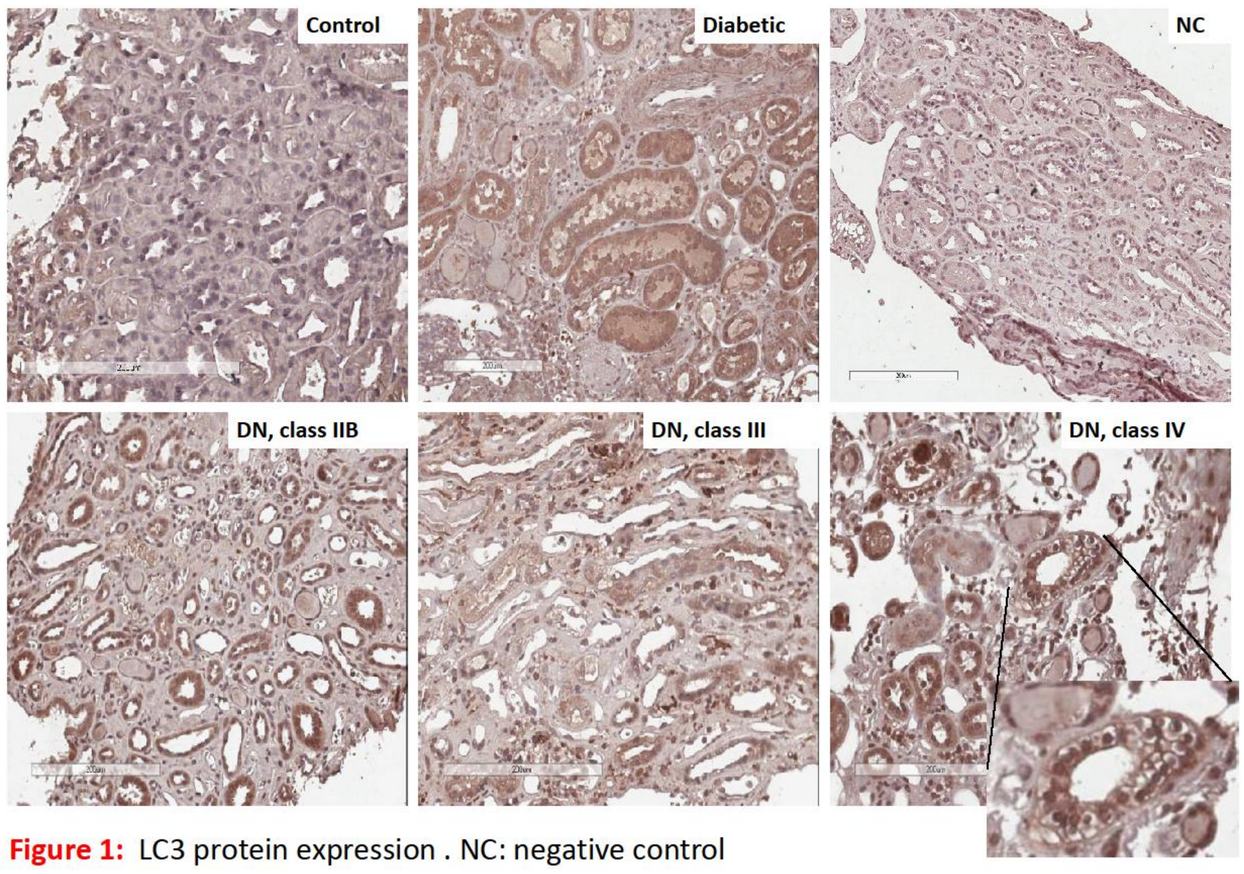

Figure 1: LC3 protein expression . NC: negative control

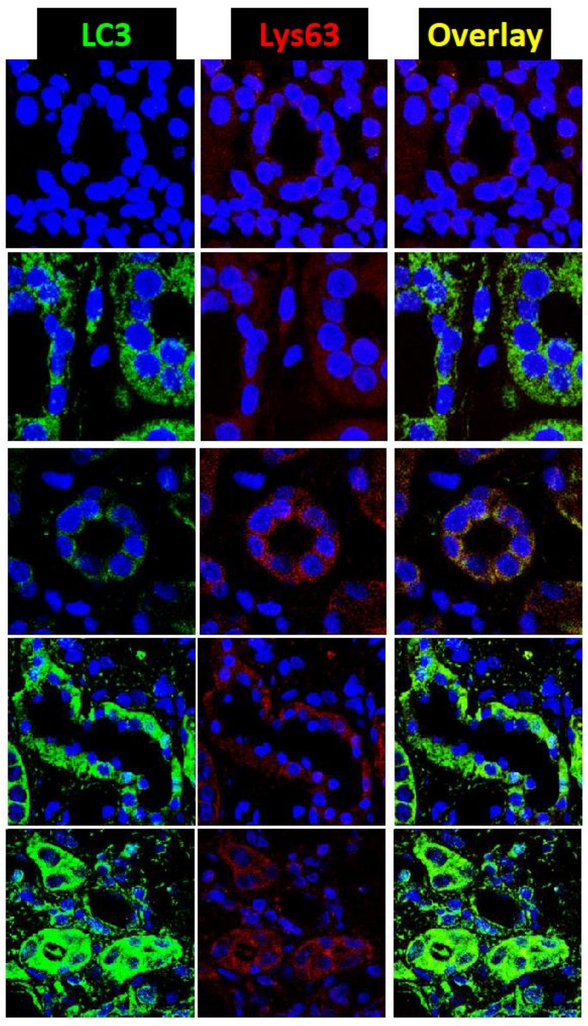

Figure 2: Immunofluorescence analysis of LC3 distribution and Lys63 ubiquitination in kidney biopsies.

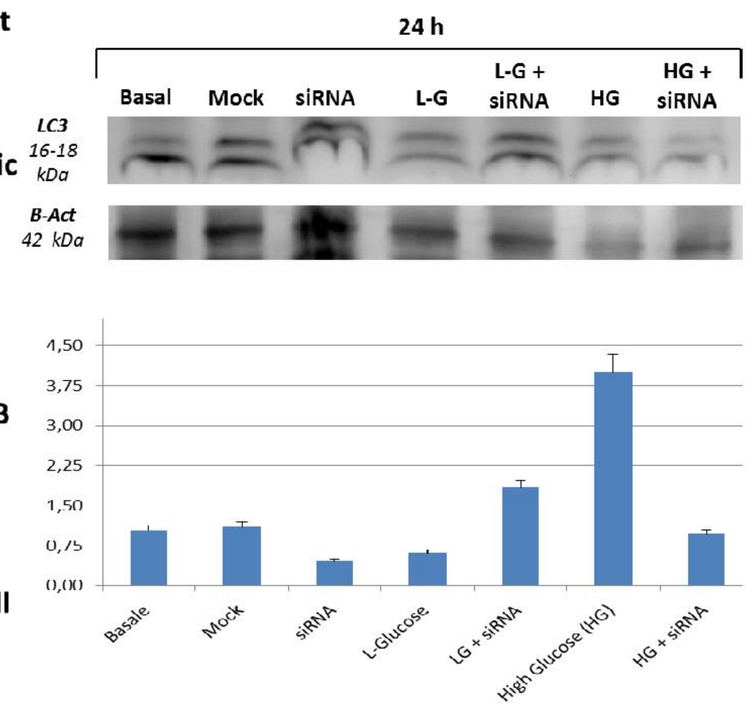

Figure 3: Effect of hyperglycemia-induced Lys63 ubiquitination on LC3 protein expression in HK2 cells. siRNA: siUBE2v1; L-G: L-glucose, control; HG: high glucose.

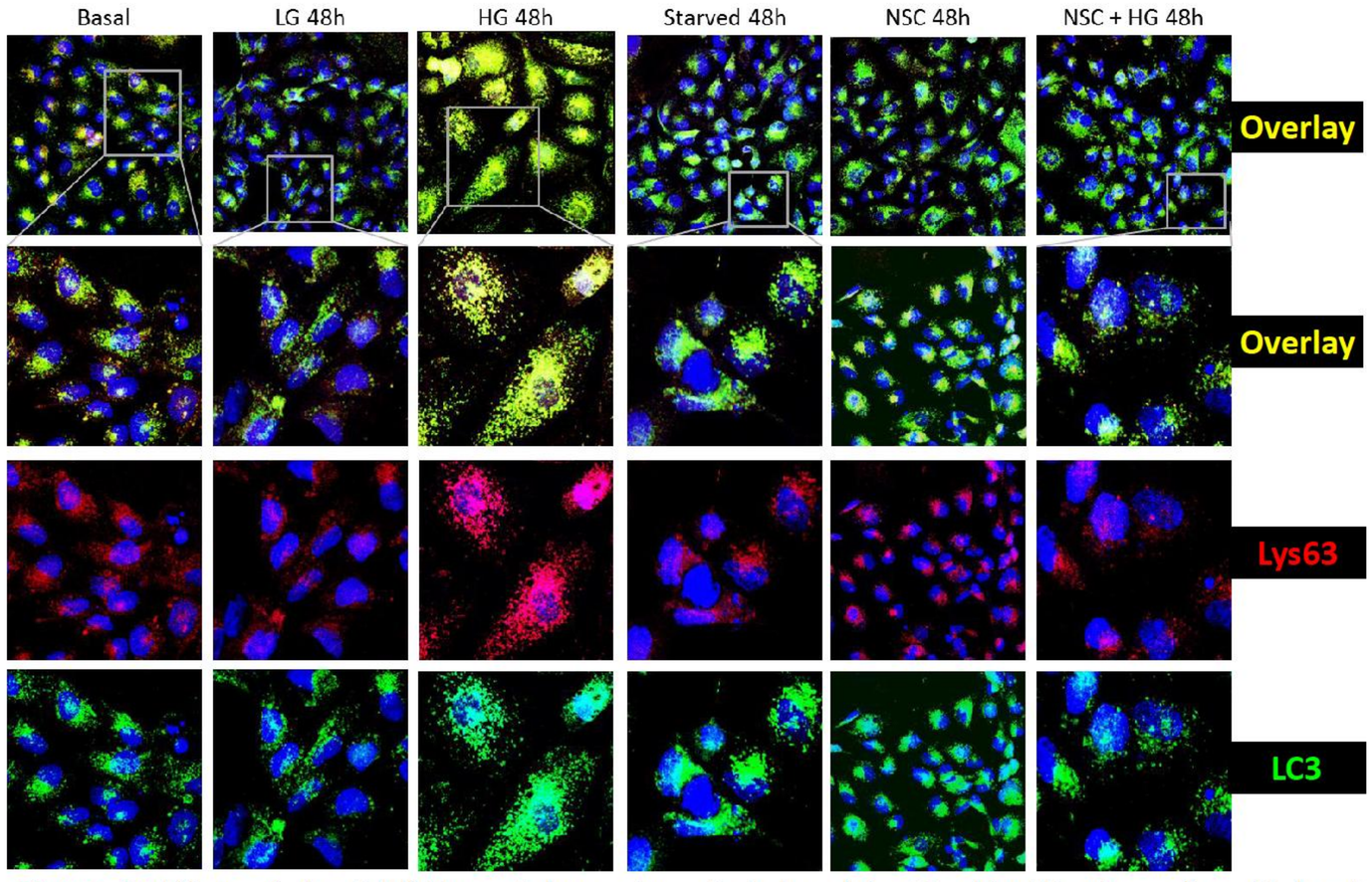

Figure 4: Effect of the inhibition of hyperglycemia-induced Lys63 ubiquitination, after 48 h of stimulation, on LC3 protein expression in HK2 cells. NSC: NSC697923 lysine63 ubiquitination specific inhibitor. Starved 48h: positive control for autophagy

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