HIGH GLUCOSE PROMOTES APOPTOSIS IN GLOMERULAR PODOCYTES BY SUPPRESSING THE INSULIN SIGNALING PATHWAY

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OBJECTIVES

Chronic hyperglycaemia, as seen in type II diabetes, results in both morphological and functional impairments of glomerular podocytes, the cells responsible for maintaining the filtration barrier [1,2]. Insulin signaling is one of the key pathways that ensure metabolic homeostasis and podocyte survival [3]. The principal aim of this study was to investigate the functionality of the insulin signaling pathway under the effects of high glucose (HG), focusing primarily on cell survival and apoptotic markers, in immortalized human glomerular cells (HGEC; podocytes) and isolated glomeruli from healthy rats.

METHODS

HGEC and isolated glomeruli were cultured for various time intervals under HG concentrations in the presence or absence of short insulin pulse. Free intracellular glucose concentration was ascertained by means of emission of fluorescence upon glucose oxidation. Protein expression of insulin signaling components (insulin receptor, IR; its phosphorylated form, p-IR; insulin receptor substrate 1, IRS1; phosphorylated Akt, p-Akt) and of apoptotic markers (Fox01,03, PARP and Caspase-3) was determined by Western blotting. Apoptosis was further confirmed by DNA laddering [4] and TUNEL assay.

RESULTS

Exposure of HGEC to HG led to:

- increased glucose uptake (Fig. 1A)
- downregulation of insulin signaling (Figs. 1B-F)
- diminished PI3K-regulated Akt phosphorylation (Figs. 1E-G)
- altered IRS-1 phosphorylation favoring serine versus tyrosine residues (Figs. 1H, I)
- increased susceptibility to apoptosis, as seen by increased cleavage of PARP/Casp3 (Figs. 2A, B), DNA fragmentation (Fig. 2C) and TUNEL staining (Fig. 2D).

Exposure of glomeruli to HG led to:

- downregulation of insulin signaling (Fig. 3A)
- induction of apoptosis (Fig. 3B)

Figure 1: High glucose results in downregulation of insulin signaling in HGEC

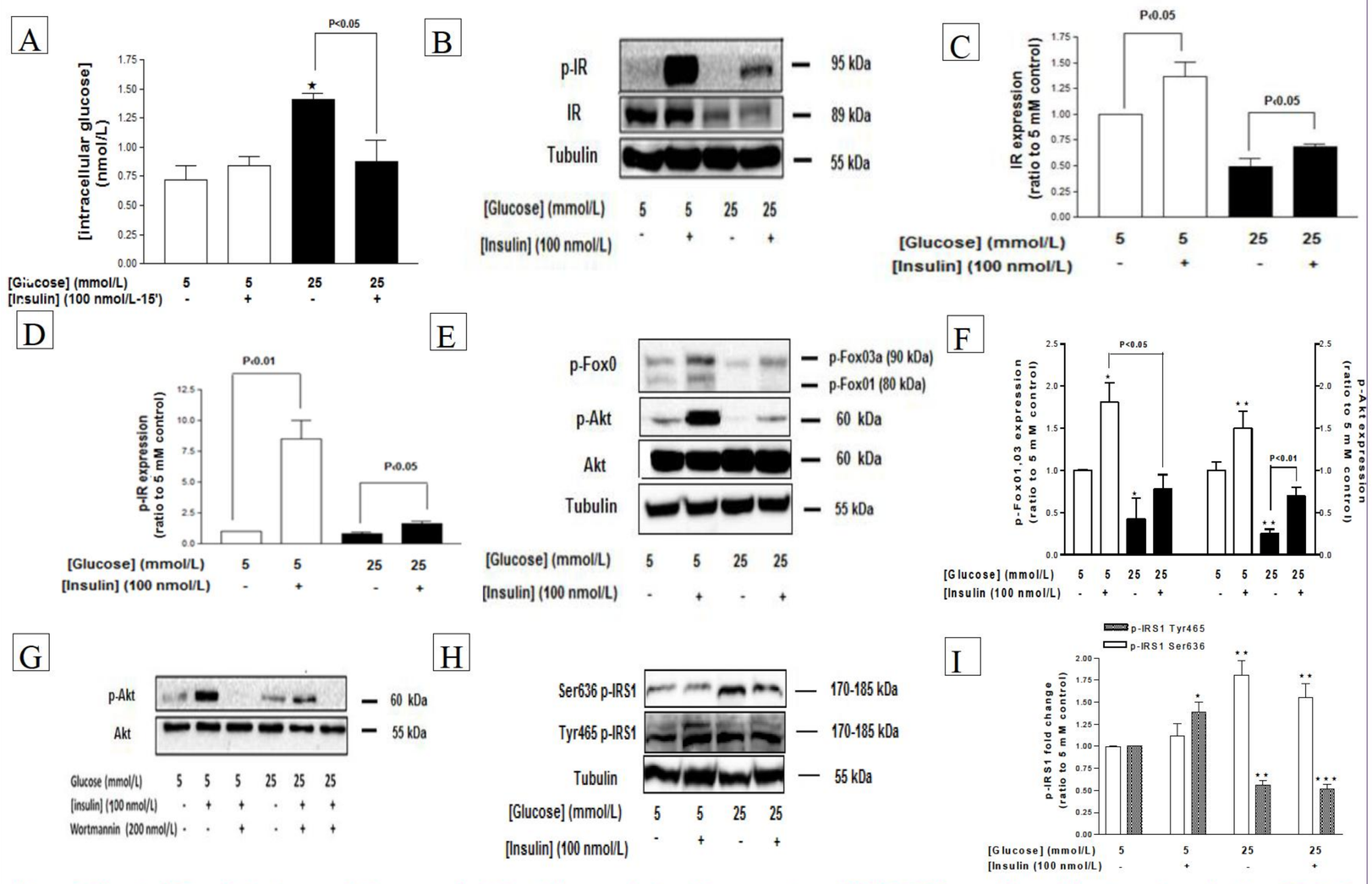

Figure 1: Chronic HG results in increased glucose uptake (A) and downregulation of the expression of IR (B, C; Western blot and densitometric analysis), p-IR (B, D; Western blot and densitometric analysis), p-Akt and p-Fox01,03 (E, F; Western blot and densitometric analyses). IRS-1 phosphorylation pattern is altered under HG (H, I; Western blot and densitometric analyses). Data represent mean SEM, n=3-5, *P<0.05, **P<0.01 vs. control (5 mmol/L glucose).

Figure 2: HG predisposes HGEC to apoptosis

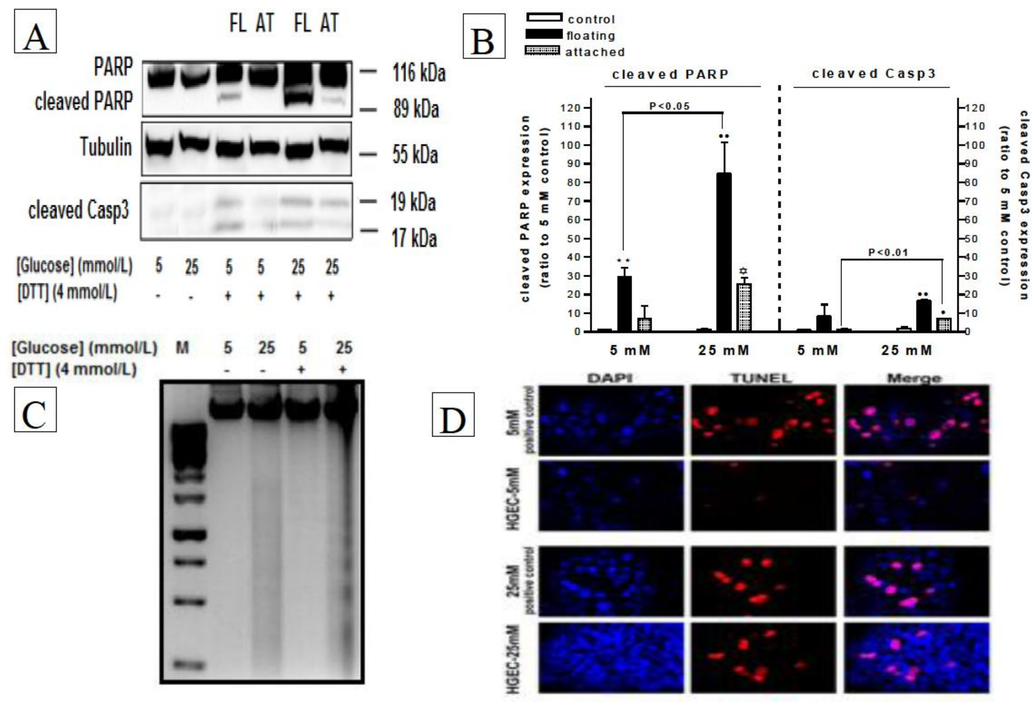

Figure 2: Treatment of HGEC with DTT for 24 h results in increased PARP and Caspase-3 cleavage, a response that occurs in a greater extent in adherent 25 mmol/L glucose-culture cells than in control (5 mmol/L glucose-cultured cells) (A, B; Western blot image and densitometric analysis; FL-floating cells, AT-attached cells). Increased apoptosis is further confirmed by DNA fragmentation analysis (C) and TUNEL staining (D). Data represent mean SEM, n=3-5, **P<0.01 vs. control (5 mmol/L glucose) and *P<0.05, **P<0.01 vs. control (25 mmol/L glucose).

Figure 3: HG impairs glomerular insulin signaling and promotes apoptosis

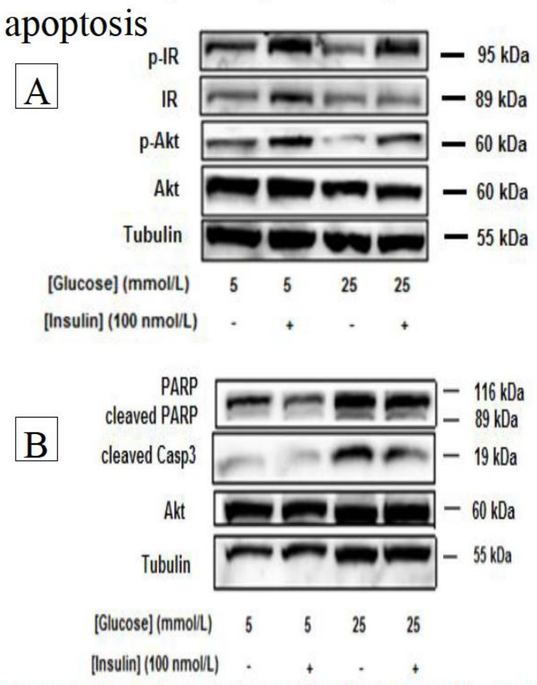

Figure 3: Treatment of glomeruli with 25 mmol/L glucose for 96 h resulted in downregulation of the p-Akt and p-IR levels, without affecting total IR levels (A; Western blot image) which coincided with enhanced apoptosis as evident by increased PARP and Casp3 cleavage (B; Western blot image). N=4-5.

CONCLUSIONS

These results suggest that HG compromises the insulin signaling pathway in the glomerulus, promoting a pro-apoptotic environment. Our data suggest a possible critical step for this malfunction at the level of IRS-1 phosphorylation. We hereby demonstrate the insulin signaling pathway as another target for investigation for the prevention and/ or treatment of diabetic nephropathy.

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