Review

Heparin interaction with a receptor on hyperglycemic dividing cells prevents intracellular hyaluronan synthesis and autophagy responses in models of type 1 diabetes

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Abstract

Previous studies and ongoing research indicate the importance of an interaction between a putative receptor on dividing cells in hyperglycemia and the non-reducing end motifs of heparin stored in mast cell secretory granules and how this interaction prevents activation of hyaluronan synthesis in intracellular compartments and subsequent autophagy. This suggests a new role for endosomal heparanase in exposing this cryptic motif present in the initial large heparin chains on serglycin and in the highly sulfated (NS) domains of heparan sulfate.

Inactive hyaluronan synthases normally migrate to the plasma membrane where they are inserted and activated. Then they alternately add cytosolic substrates, UDP-glucuronate (UDP-GlcUA) and UDP-*N*-acetyl-D-glucosamine (UDP-GlcNAc), onto the reducing end of the elongating hyaluronan chain that is being extruded into the extracellular matrix [1,2]. Hyperglycemic mesangial cells stimulated to divide from G0/G1 to S phase activate hyaluronan synthases by a PKC pathway in intracellular membrane compartments – endoplasmic reticulum (ER), Golgi and transport vesicles – in response to the elevated cytosolic UDP-substrate concentrations due to glucose stress. The abnormal deposition of the polyanionic hyaluronan macromolecules into the ER induces ER stress, autophagy and subsequent extrusion of an extracellular monocyte-adhesive hyaluronan matrix after completing cell division [1,3,4].

This mechanism occurs in kidneys of streptozotocin-induced type 1 diabetic rats. Mesangial cells in the hyperglycemic kidney glomeruli initiate cell division within the first week with subsequent autophagy and continuous accumulation of an extracellular hyaluronan matrix, Fig. 1. This is accompanied by an influx of monocytes/macrophages that initiate pro-inflammatory and fibrotic responses that lead to nephropathy, proteinuria, weight loss and kidney failure by 6 weeks [4,5].

Previous studies showed that daily IP injection of a small amount of heparin in this diabetic rat model prevented the pathological responses and sustained kidney function for at least 8 weeks [6,7]. To address the mechanisms involved, hyperglycemic mesangial cells were treated with a low concentration (2 μg/ml) of heparin after initiating progression from G0/G1 to S phase of cell division [5]. This prevented the activation of the hyaluronan synthases in the

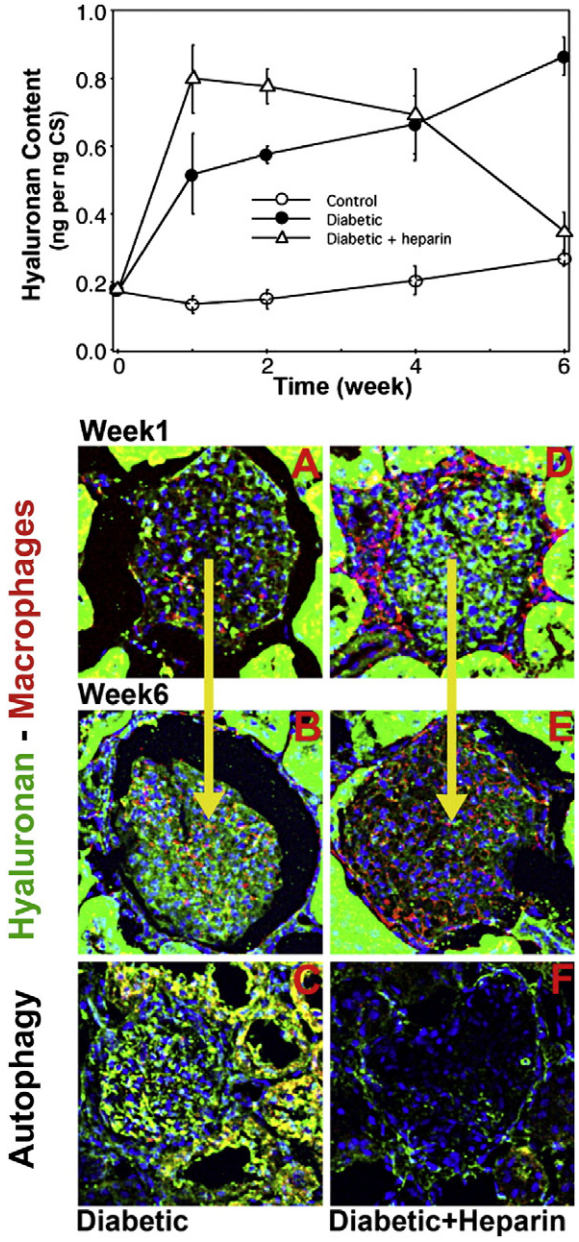

Fig. 1. HA concentrations in glomeruli isolated from kidneys 1–6 weeks after initiating diabetes in rats treated without or with heparin (IP) are shown in the upper panel. Sections of glomeruli from 1 and 6 week kidneys of diabetic rats (A–C) and diabetic rats treated with heparin (D–F) were stained for hyaluronan (green) and macrophages (red) (A, B, D, E), and for markers of autophagy, LC3 (green) and cyclin D3 (red) (C, F). Modified from figures in reference [5].

intracellular membrane compartments and allowed the cells to complete cell division without the ER stress and autophagy responses. However, it also re-programmed the cells to address the high glucose stress after completing cell division by inducing a large hyaluronan synthesis response at the plasma membrane with subsequent extracellular extrusion of an extensive monocyte-adhesive hyaluronan matrix.

This mechanism appears central to the glomerular responses in kidneys in heparin-treated diabetic rats [5], Fig. 1. Mesangial cells in glomeruli showed no autophagy responses over the 6-week period studied. Within the first week, the glomeruli accumulated an extensive extracellular hyaluronan matrix around the mesangial cells at which time there were few macrophages present in the glomeruli. By 6 weeks there were extensive numbers of macrophages in the glomeruli, and the hyaluronan matrix was reduced to near control levels. This provides evidence that the mesangial cells in the heparin-treated diabetic rats initiate and sustain a large synthesis of extracellular hyaluronan matrix to address the glucose stress while maintaining kidney function, and that the recruited macrophages in the glomeruli effectively remove the hyaluronan matrix without inducing mesangial cell inflammatory and fibrotic responses.

Fluorotagged heparin was used to track the binding and subsequent entry of the heparin into the dividing hyperglycemic cells [5]. It bound onto the cell surface within 1 h after initiating cell division, was intracellular by 2 h, and localized in the ER, Golgi and nuclei by 4 h. Exposure of the cells to heparin for only 4 h was sufficient to prevent the intracellular hyaluronan and autophagy responses, and to reprogram the cells to synthesize the extracellular monocyte-adhesive hyaluronan matrix after completing division. These results provide strong evidence that a cell surface receptor is involved in order to bind, internalize and transport the large (~15 kDa), highly polyanionic heparin to these intracellular compartments. Further, heparin interaction with this receptor would initiate activation of the intracellular pathways that: 1) inhibit the PKC activation of the hyaluronan synthases in the intracellular compartment membranes, and 2) initiate the mechanism that induces the synthesis of the extensive extracellular hyaluronan matrix after completing cell division.

How does heparin do this? Heparin is synthesized by mast cells as the proteoglycan serglycin, which contains ~15 long heparin chains (~100 kDa) on a repetitive serine–glycine stretch in the core protein, Fig. 2 [8]. However, during or after its progression to the storage granules, the heparin chains are hydrolyzed into much smaller fragments (~15 kDa) by heparanase. This is the anti-coagulant form of heparin that is in extensive clinical use. There is only one heparanase in our genome, and it hydrolyzes the original intact heparin chain in regions that expose non-reducing terminal, GlcNS,-*O*-S residues on many of the fragments, Fig. 2, which is in good agreement with a previous report (see Table 1 in ref.