Reversal by Growth Hormone of Homocysteine-induced Epithelial-to-Mesenchymal Transition through Membrane Raft-Redox Signaling in Podocytes

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ABSTRACT

Epithelial-to-Mesenchymal Transition (EMT) is an important pathological mechanism mediating glomerular injury or sclerosis in a variety of renal and systemic diseases such as hyperhomocysteinemia (Hcy). The present study was designed to test whether Hcy-induced EMT in podocytes is reversed by growth hormone (GH), a hormone regulating cell proliferation and growth and to explore the critical and molecular mechanism mediating its action. It was found that Hcy-induced significant EMT in podocytes, as shown by marked decreases in slit diaphragm-associated protein P-cadherin and zonula occludens-1 as epithelial markers and by dramatic increases in the expression of mesenchymal markers, FAP-1 and α-SMA. When podocytes were treated with GH at 25 ng/mL, however, Hcy failed to induce podocyte EMT. Using electrophysiological spin response methodology, Hcy-induced supernatant (Hcys) production via NOX activation was found to be significantly inhibited by GH (86%). Functional studies in Hh were shown to substantially inhibit Hcy-induced increases in the permeability of podocyte monolayers and to block the decrease in protein expression in these cells. In addition, NOX subunit, gp91phox and NOX receptors aggregated in nuclear membrane (MR) clusters, which produced O2• − in response to Hcy and could be blocked by GH, MR disruptors fluphen and MC1 or NOX inhibitors, apocynin. It is concluded that Hcy-induced podocyte EMT is associated with transmembrane MR-redox signaling and that GH reverses this Hcy-induced EMT protecting podocytes from functional disturbance (supported by NIH grants DK659927, HL57244 and HL75316).

METHODS

Cell culture. Conditionally immortalized mouse podocyte cell line were cultured on collagen I-coated flasks or plates in RPMI 1640 medium supplemented with 10% fetal bovine serum, 10 amulet recombinant mouse insulin at 37°C. After differentiation at 37°C for 10–14 days without interferon-γ, podocytes were used for the following experiments.

RESULTS

Background. Previous studies from our laboratory have demonstrated that EMT occurs in podocytes during IL-6 and enhanced EMT may result in podocyte dysfunction. Not activation and subsequent oxidative stress is a common mechanism mediating Hcy-induced EMT in podocytes.

Growth hormone (GH) has been demonstrated to decrease oxidative stress and recover antioxidant defenses via reduced cellular ROS generation through various pathways such as upregulation of the expression of Alke-SOD, Cu, Zn-SOD, GPx1 and EM2 in endothelial cells.

A growth hormone-releasing peptide, ghrelin has also been found to have protective effects from Hcy-induced coronary endothelial dysfunction by increasing expression of endothelial nitric oxide synthase and reducing local oxidative stress. However, it remains unknown whether GH is able to protect podocytes or glomeruli from Hcy-induced injury.

The hypothesis in the present study is that GH protects podocytes from Hcy-induced injury through attenuation of enhanced EMT associated with NOX activation.

CONCLUSION

• GH improves Hcy-induced EMT in podocytes and thereby protects podocytes from dysfunction or injury through membrane cell-redox signaling.

• GH may be used as a new therapeutic remedy to prevent the progression of end-stage renal disease during Hcy due to aging, hypertension, chronic kidney disease or other diseases.