Lipid Raft Redox Signaling Platform and Apoptosis of Podocytes upon Homocysteine Stimulation

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ABSTRACT

Lipid raft (LR) redox signaling platforms associated with NADPH oxidase (NOX) were reported to mediate the actions of death receptor activation in different cells. It is interesting to know whether this LR redox signaling platform is also involved in podocytes injury during hyperhomocysteinemia. The present study first characterized the presence of the NOX membrane subunit gp91phox and its LR clusters in podocytes, and then tested whether this redox signaling platform contributes to hyperhomocysteinemia (Hiys)-induced podocytes apoptosis. It was found that Hcys markedly increased the expression of gp91phox and stimulated NOX-dependent superoxide (O2-) production in a concentration-dependent manner, as measured by electronic spin resonance (ESR). Using confocal microscopy, gp91phox was found to aggregate in LR clusters upon Hcys stimulation, which was inhibited by lipid raft disruptors, methyl-β-cyclodextrine (MCD) and filipin. Functionally, increased O2- production associated with these LR-gp91phox platform was also blocked by MCD or filipin. Flow cytometry showed that Hcys induced podocytes apoptosis, which could be attenuated by gp91phox siRNA or LR disruptors. Our results indicate that the formation of gp91phox-associated LR redox signaling platform importantly contributes to podocytes injury during exposure to high Hcys levels (supported by NIH grants DK64987, HL70536, and HL71444).

METHODS

Cell culture. Conditionally immortalized mouse podocyte cell line was cultured on collagen I-coated flasks or plates in RPMI 1640 medium supplemented with 10% fetal bovine serum, 10 unit/ml recombinant mouse interferon-γ (MCD) and filipin. Functionally, increased O2- production associated with these LR-gp91phox platform was also blocked by MCD or filipin. Flow cytometry showed that Hcys induced podocytes apoptosis, which could be attenuated by gp91phox siRNA or LR disruptors. Our results indicate that the formation of gp91phox-associated LR redox signaling platform importantly contributes to podocytes injury during exposure to high Hcys levels (supported by NIH grants DK64987, HL70536, and HL71444).

RESULTS

Podocytes are highly differentiated glomerular epithelial cells which lie in the outmost of the glomerular filtration barrier and compose the most important structure in preventing the leakage of plasma proteins into urine.

Podocytes injury is an important early event initiating glomerular sclerosis. Our previous studies have demonstrated that NADPH oxidase (NOX) importantly contributes to the development of glomerular sclerosis associated with hyperhomocysteinemia (Hiys). However, the effects of Hiys on podocytes and the role of NOX in this process are still elusive.

Lipid raft (LR) redox signaling platforms associated with NOX have been reported to mediate the actions of death receptor in different cells. It is interesting to know whether this LR redox signaling platform is also involved in podocytes injury induced by Hcys.

Our hypothesis is that Hcys can induce podocytes injury through activation of NOX which was recruited by lipid-raft clustering. In the present study, we first characterized the presence of two common redox signaling components, the NOX membrane subunit gp91phox, as well as cytosolic subunit-p47phox in podocytes, and then tested whether this redox signaling mechanism contributes to podocytes injury and subsequent apoptosis during hyperhomocysteinemia stimulation.

CONCLUSION

Hiys-induced podocytes apoptosis was effectively inhibited by gp91phox siRNA or LR disruptors. This suggests that inhibition of the gp91phox-associated LR redox signaling platform limits podocytes injury. Further, our findings suggest that Hcys may induce lipid raft clustering and the recruitment of NOX subunits to these lipid raft platforms.