Podocyte Specific Deletion of Acid Ceramide Predisposes Mice to Obesity-Induced Glomerular Injury

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ABSTRACT

Acid ceramidase (AC) is a key regulatory enzyme involved in ceramide metabolism, playing a critical role in the intercellular lipid metabolism and molecular trafficking. Given recent reports that obesity enhances ceramide production, leading to the activation of NADPH oxidase and consequent development of glomerular sclerosis, the present study was designed to test whether AC contributes to the development of glomerular injury associated with obesity. Here, we generated and characterized the podocyte-specific AC knockout mice by crossing fosfomethyl AC-cre and Neph+cre promoter mice. The podocyte specific AC heterozygous (AC+/Neph+) and wild type (AC+/+ mice were fed a high fat diet (HFD) or normal chow (ND) for 12 weeks to produce obesity. Immunohistochemical analysis demonstrated that AC expression was reduced in glomeruli of AC+/Neph+ mice, and increased in AC+/+ mice. In contrast, the ceramide level, and lesion expression were higher in glomeruli of AC+/Neph+ than AC+/+ mice. Furthermore, Western blot analysis of glomerular tissues showed that IFP significantly decreased the AC expression in AC+/- mice, but not in AC+/+ mice. Consequently, the urinary protein excretion was significantly higher in HFD fed AC+/Neph+ than AC+/+ mice. In an in vitro study of podocytes, AC inhibitor, SNAP-006 decreased the AC expression, VEGF level and increased the disease expression compared to control cells. In conclusion, our observations reveal that normal expression of AC contributes to the development of podocyte injury and the defect of this gene expression is a critical mechanism triggering podocyte injury and ultimately resulting in obesity-associated end-stage renal disease (Supported by NIH grants DK82077 and DK010651).

METHODS

Animals: Eight-week-old male AC- and podocyte specific acidic ceramide hydrolase (AcNH)-/- mice were used in the present study. Mice were treated with either a normal diet or high fat diet for 12 weeks.

Morphological examinations: The fixed kidney were paraffin-embedded, sections were prepared and stained with periodic acid-Schiff (PAS). Glomerular injury index was calculated from 1 to 4 on the basis of the degree of glomerulosclerosis and mesangial matrix expansion as described previously. Glomerulosclerosis was scored for 100 glomeruli in each kidney slice under microscope, when each glomerulus was graded level 0-6, 0: no lesion, 1: represents sclerosis of <5% of the glomerular area; 2+, 3+, 4+ represents sclerosis of 5-9%, 10-19%, >20% of the glomerular area, respectively. The kidney injury index was then calculated by summing the scores from counted glomeruli. The immunohistochemical analysis of renal tissue was performed to detect acid ceramidase and ceramide staining using anti-acid ceramidase (Santa Cruz, 1:800 dilution) and anti-AcNH (Alenxas, 1:50 dilution) antibodies.

Urinary total protein and albumin excretion measurement: The 24-hour urine samples were collected using metabolic cages and subjected to total protein and albumin excretion measurements. Total protein content in the urine was detected by Bradford method using a UV spectrophotometer. Urine albumins were detected by using a commercially available mouse albumin enzyme-linked immunosorbent assay kit (Bethyl Laboratories, Montgomery, TX, USA) as described previously [1,2].

Immunofluorescence staining of cells: Cells were fixed in 4% PFA, and then blocked with 5% BSA for 60 min. After incubated with primary antibodies overnight, the cells were stained with Alexa-567 Fab-globular secondary antibodies. After washing, the cells were mounted with DAPI-containing mounting solution. Then, the slides were observed under a fluorescent microscope and images were taken [2].

ESR detection of superoxide (O2-) production: NADPH-dependent O2 production were measured by ESR measurement [1].

ELISA for vascular endothelial growth factor (VEGF-A): Podocytes were treated with AC inhibitor or vehicle for 12 h. After the treatment, the supernatant was collected for ELISA assay of VEGF-A using a commercially available kit (R&D system, Minneapolis, MN).

RESULTS

Acid ceramidase (N-acetyl-sphingomeline deacylase, AC) is the lipid hydrolase responsible for the degradation of ceramide into sphingosine and free fatty acids with in the lysosomes. AC not only hydrolyzes ceramide into sphingosine but also can synthesize ceramides from sphingosine and free fatty acids in vitro and in vivo.

Acid ceramidase (AC) is a key regulatory enzyme involved in sphingoid metabolism, and plays a role in the pathology of several diseases such as Parkin disease, diabetes, Alzheimer disease, obesity and cancer.

Recent reports showed that obesity enhances ceramide production, leading to the activation of NADPH oxidase and consequent development of glomerular sclerosis. However, the role of acid ceramidase in the renal glomerular function remains elusive.

Hence, the present study was designed to test whether acid ceramidase contributes to the development of glomerular injury associated with obesity.

Fig. 1: Acid Ceramidase expression were determined by real time PCR ( panel A and B). Western blot (panel C), and immunohistochemical (panel D) analysis. The acid ceramidase expression was significantly lower in glomeruli of high fat diet fed mice compared to normal diet fed mice. These results suggest that acid ceramidase is expressed in podocytes of AC+/Neph+ wild type mice with significant expression in AC+/+ mice, and significantly less expression in AC+/Neph+ mice. High fat diet treatment significantly increased the glomerular damage index in AC+/Neph+ mice compared to AC+/+ mice. High fat diet treatment significantly increased the glomerular damage index in AC+/Neph+ mice compared to AC+/+ mice fed a normal diet. * significant difference (P<0.05) from AC+/+ mice fed ND. # significant difference (P<0.05) from HFD treated AC+/+ mice.

Fig. 2: A) Podocyte specific acidic ceramide hydrolase mice were generated using PCR. Reaction of a PCR product at 550 bp induces AcNH-/-, a PCR product of 462 bp indicates AcNH+/- mice while PCR product of 100 bp indicates Cre gene. B and C: The acid ceramidase expression was dramatically diminished in podocyte specific acidic ceramide hydrolase mice (AcNH+/Neph+) compared to AcNH+ mice. After transtion of the high fat diet further increased the AC in both AcNH+ and podocyte specific Ac NH hydrolase mice. In contrast, ceramide expression was dramatically increased in AcNH+ mice compared to AcNH+ mice.

RESULTS

Background

Ac acid ceramidase deficiency promotes podocyte injury and ultimately results in obesity-associated renal disease.

Fig. 3: Urinary total protein and albumin excretion in podocyte specific acidic ceramide hydrolase mice with or without high fat diet treatment. The 24-hour urinary total protein and albumin excretion were significantly higher in AcNH+/Neph+ mice compared to AcNH+ mice. However, the HFD treatment significantly enhanced the urinary total protein and albumin excretion in AcNH+/Neph+ mice, but not in AcNH+/+ mice. * significant difference (P<0.05) from AcNH+/+ mice fed ND. # significant difference (P<0.05) from HFD treated AcNH+ mice.

Fig. 4: Glomerular injury was more pronounced in AcNH+ mice. Photomicrographs (panel A) show typical glomerular structures in normal diet or high fat diet treated AcNH+/+ and AcNH+ mice. Panel B depicts semi-quantitative score of glomerular damage index (0-6). Morphological analysis showed that AcNH+ mice had more glomerular damage compared to AcNH+/+ mice. Glomerular damage index was significantly higher in AcNH+ mice compared to AcNH+/+ mice. High fat diet treatment significantly increased the glomerular damage index in AcNH+ mice compared to AcNH+/+ mice fed a normal diet. * significant difference (P<0.05) from AcNH+/+ mice fed ND. # significant difference (P<0.05) from HFD treated AcNH+ mice.

Fig. 5: Immunohistochemical analysis revealed that podocyte injury was more pronounced in podocyte specific AC hydrolase mice. Typical images of podocyte (left panel) or glomerulus (middle panel) staining in glomeruli from AcNH+/+ and AcNH+ mice with or without HFD treatment. High fat diet treatment decreased the podocyte staining and increased the amount of staining in AcNH+ mice (B and C). Under transmission electron microscopy, the intact structures of podocyte foot processes shown in glomeruli of mice on the ND were destroyed in AcNH+ mice, as shown by evident foot process effacement in AcNH+ mice with or without HFD (right panel). ND, Normal diet, HFD, High fat diet. These data reveal that lack of AC gene induces the podocytes damage in glomeruli.

Fig. 6: Local oxidative stress was increased in the glomeruli of AcNH+ mice. NADPH dependent O2 production was measured by ESR spectrophotometer. Summarized data demonstrated that the glomeruli O2 production was increased in podocyte AcNH+ mice compared to AcNH+ mice. Superoxide radicals that mice lacking acid ceramidase induce the local oxidative stress in the kidney. * significant difference (P<0.05) from AcNH+ mice fed ND. # significant difference (P<0.05) from HFD treated AcNH+ mice.

Fig. 7: Effect of acid ceramidase inhibitor (SNAP-006) on podocyte injury and VEGF production in cultured podocytes. Podocytes were treated with AC inhibitor for 12 h and measured the protein expression by ESR/PCR and indirect immunofluorescence method. The acid ceramidase inhibition decreased the podocyte expression compared to control cells (Panel A and B). In addition, VEGF expression in podocytes was markedly decreased by ELISA. This inhibition decreased the VEGF production compared to control cells (Panel C). These results suggest that lack of AC has a functional damage in podocytes. * significant difference (P<0.05) from control group.

CONCLUSION

The observations reveal that normal expression of AC contributes to the function of podocytes and the defect of this gene expression in a critical mechanism triggering podocyte injury and ultimately resulting in obesity-associated renal injury.

REFERENCES
