The American Journal of Pathology, Vol. 190, No. 6, June 2020

The American Journal of
PATHOLOGY
ajp.amjpathol.org

See related article on page 1211

COMMENTARY

Podocytes

The Role of Lysosomes in the Development of Nephrotic Syndrome

Marica Giliberti, Adele Mitrotti, and Loreto Gesualdo

From the Nephrology Unit, Department of Emergency and Transplant Organs, University Aldo Moro of Bari, Bari, Italy

Podocytes are terminal, visceral epithelial cells that lack proliferative properties, making intracellular homeostasis essential for their integrity. Podocytes are characterized by interdigitating foot processes. Secondary foot processes surround glomerular basement membrane and together with the glomerular basement membrane and endothelial cells generate the glomerular filtration barrier, regulating the protein traffic through our kidneys. Podocytes are critical for the maintenance of glomerular morphology and function. Furthermore, they represent a frequent target of injury, as it happens in case of genetic mutations, immune disorders, infections, hemodynamic defects, toxic exposure, or metabolic alteration.[1]

Congenital or acquired alterations in podocytes are frequently involved in different kidney diseases, including minimal change disease, focal segmental glomerulosclerosis, membranous nephropathy, Fabry disease, diabetic nephropathy, HIV-associated nephropathy, lupus nephritis, and preeclampsia.[1–3] These conditions are mostly characterized by proteinuria and eventually by a full nephrotic syndrome.

Lysosomes represent an essential intracellular component with digestive and recycling properties.[4] Lysosomes are critical for cellular activity in podocytes,[5] due to their scavenging ability, as demonstrated by the emerging knowledge that lysosome dysfunction may relate with glomerular disease.

Mutations in genes encoding for lysosomal proteins or proteins involved in lysosomal activity are known causes of genetically determined nephropathies, such as Fabry disease, Tay-Sachs disease, cystinosis, and Nieman-Pick disease. *ASAH1* encodes for lysosomal acid ceramidase (AC) and *ASAH1* mutations in humans cause autosomal recessive disorders, such as Farber lipogranulomatosis and spinal muscular atrophy with progressive myoclonic epilepsy.[6–8]

In this issue of *The American Journal of Pathology*, Li et al[9] demonstrated that ceramide accumulation in podocytes may lead to cellular damage and nephrotic syndrome, in mice. A knockout mouse strain ($Asah1^{fl/fl}/Podo^{Cre}$) with a podocyte-specific deletion of the α subunit (main catalytic subunit) of lysosomal acid ceramidase (AC) was used in this study. Ceramide accumulation, determined by liquid chromatography–tandem mass spectrometry, was demonstrated in isolated glomeruli of $Asah1^{fl/fl}/Podo^{Cre}$ mice compared with their littermates.[9]

Ceramides are lipids belonging to the family of sphingolipids. They are converted in sphingomyelin first and then sphingolipids, the main component of plasma membrane. Plasma membranes are usually catabolized into lysosomes, where ceramides are generated from degradation of sphingolipids and then regenerated. The interest in the molecular biology and ceramide metabolism is due to the recognized link between cellular clearance dysfunction and the pathogenesis of some lysosome-related disorders.[10]

AC is responsible for the hydrolysis of ceramide into sphingosine and free fatty acid,[11] and it may have a role in the lysosomal system. Ceramide plays a central role in metabolism of other cellular sphingolipids; therefore,

Accepted for publication April 8, 2020.
Disclosures: None declared.

Address correspondence to Loreto Gesualdo, M.D., F.E.R.A., Nephrology, Dialysis and Transplantation Unit, Aldo Moro University of Bari, Azienda Ospedaliero-Universitaria Consorziale Policlinico, Piazza Giulio Cesare, 11 - 70124 Bari, Italy. E-mail: loreto.gesualdo@uniba.it

Copyright © 2020 American Society for Investigative Pathology. Published by Elsevier Inc. All rights reserved.
https://doi.org/10.1016/j.ajpath.2020.04.001

alterations in ceramide metabolism may result in different pathologies.

The synthesis and accumulation of ceramide in response to cellular stress is known to mediate cancer cell death through various mechanisms, including apoptosis and autophagy. Enzyme ceramidase may represent a therapeutic target of cancer.[12–14] In fact, AC is overexpressed in a variety of cancer cell lines, and the enzymatic inhibition by novel specific inhibitors or other traditional cancer agents, alone or in combination, induces apoptosis in cancer cells.[15–17]

Farber disease is a rare lysosomal storage disorder in which the genetic mutation leads to decreased AC activity with ceramide accumulation, resulting in pathologic manifestations. Farber disease is typically based on the cardinal triad symptoms: subcutaneous nodules, joint pain, and voice hoarseness. These patients present enlarged liver and spleen along with neurologic and respiratory complications.[18] However, any activity of ceramidase has not been recognized in kidney.

Although intracellular ceramide protects against neoplasm development through the induction of cell death, studies have shown that the accumulation of ceramide in cells can be involved in development of diabetic complications. Ceramide, in fact, inhibits several intracellular insulin pathways[19]: it activates a mitochondrial factor, the protein phosphatase 2A, able to inactivate Bcl-2, which is fundamental to controlling survival and apoptosis.[20] Moreover, emerging lines of evidence indicate that renal lipid dysregulation is one of the factors responsible for the development of chronic kidney disease secondary to diabetic nephropathy.[21,22]

AdipoRon *in vitro*, an orally active synthetic adiponectin agonist, binds both adiponectin receptors 1/2 and ameliorates particularly kidney diabetic complications. AdipoRon lowered cellular ceramide levels by activation of AC, which normalized ceramide to sphingosine-1 phosphate; it may prevent lipotoxicity in the kidney, particularly in both glomerular endothelial cells and podocytes. This process is mediated through an improvement in lipid metabolism and further prevents deterioration of renal function.[23] The pathogenicity of ceramide in podocytes is the main topic investigated by Li et al.[9] They suggest that the ceramide accumulation in podocytes can cause cellular damage and nephrotic syndrome. Their model showed high proteinuria and albuminuria, unresponsive to the administration of the steroids. Although no alterations were observed in the glomeruli analyzed by light microscopy classic foot process effacement and microvillus transformation was observed in podocytes of 8-week−old mice using electron microscopy. It is unclear why the podocyte morphology remains unchanged in the 4-week−old mice. It is possible that the amount of ceramide accumulation in 4-week−old podocytes, due to AC mutation, is not toxic enough to generate podocyte dysfunction at this stage. Another possibility is that, in the early stage of development, podocytes still hold modular ability, temporarily keeping structural adaptation capacity. Further studies are required to investigate these aspects.

Normally, acid sphingomyelinase catalyzes the hydrolysis of the sphingomyelin, producing ceramide. Li et al[6] also found a protective role of acid sphingomyelinase deletion in a knockout mouse cell line with a podocyte-specific deletion of the α subunit of acid ceramidase.

The use of AdipoRon in diabetic nephropathy reduces the levels of ceramide in podocytes.[23] The pathogenetic hypothesis of nephrotic syndrome onset secondary to accumulation of ceramide in podocytes is convincing, although it needs further examination.

If the relationship between ceramide accumulation and the development of some forms of nephrotic syndrome is confirmed, it would be useful to investigate *Asah1* variants, together with the other known genes responsible for nephrotic syndrome, through next-generation sequencing or whole generation sequencing techniques.

Should the hypothesis of Li et al[9] be true, it will open new diagnostic and pathogenetic definitions for the so-called idiopathic steroid-resistant nephrotic syndromes, in the era of precision medicine, and will offer new powerful therapeutic possibilities.

Furthermore, linking nephrotic syndrome to lysosomal storage disorder represents a new paradigm of research in which new insights may be born. However, future treatment strategies, such as the use of enzyme replacement therapies, would need to be investigated.

Enzyme replacement therapy is currently the standard of care for several lysosomal storage disorders: Gaucher disease, Pompe disease, Fabry disease, mucopolysaccharidosis (MPS) I, II, and VI, neuronal ceroid lipofuscinosis type 2, and Niemann-Pick B.

In Fabry disease, podocytes have been implicated because of the accumulation of globotriaosylceramide. Proteinuria is also present in Fabry disease, and it is not responsive to steroid therapy; it is usually subnephrotic.[24] This highlights the important role of lysosomes in the development of kidney podocyte damage.

The role of lysosomes in maintaining the integrity of podocytes is evidenced by recent studies on rituximab, a chimeric human IgG1 anti-CD20 monoclonal antibody with significant activity against $CD20^+$ B cells. This monoclonal medication has been adopted from the treatment of hematological diseases, and it has been successfully used in the treatment of some glomerulonephritides and nephrotic syndrome conditions, with a probably immunologic pathogenesis. The first aim of rituximab is to recognize CD20 on B lymphocytes, but it might also bind sphingomyelin−phosphodiesterase acid−like 3b and regulate acid-sphyngomyelinase activity.[25]

In conclusion, podocytes are complex cells whose alterations may result in the pathologic loss of protein in the urine. Mutations implicated in nephrotic syndrome in children and adults largely include genes encoding the structural proteins of podocytes. Lysosomal disorders, characterized by the accumulation of metabolites like ceramide, may be part of podocyte-related diseases. Investigation of the glomerular genomic background in the patients affected by

lysosome disorder may provide new interesting insights in kidney diseases. This opens the door not only to new pathogenic scenarios of nephrotic syndromes but above all therapeutic outlines, in terms of both enzyme replacement therapy and targeted gene therapy.

References

1. Wiggins RC: The spectrum of podocytopathies: a unifying view of glomerular diseases. Kidney Int 2007, 71:1205−1214
2. Watanabe A, Feltran LS, Sampson MG: Genetics of nephrotic syndrome presenting in childhood: core curriculum 2019. Am J Kidney Dis 2019, 74:549−557
3. Jefferson JA, Nelson PJ, Najafian B, Shankland SJ: Podocyte disorders: core curriculum 2011. Am J Kidney Dis 2011, 58:666−677
4. Li G, Kidd J, Li PL: Podocyte lysosome dysfunction in chronic glomerular diseases. Int J Mol Sci 2020, 21:1559
5. Pavenstädt H, Kriz W, Kretzler M: Cell biology of the glomerular podocyte. Physiol Rev 2003, 83:253−307
6. Park JH, Schuchman EH: Acid ceramidase and human disease. Biochim Biophys Acta 2006, 1758:2133−2138
7. Sugita M, Dulaney JT, Moser HW: Ceramidase deficiency in Farber's disease (lipogranulomatosis). Science 1972, 178:1100−1102
8. Zhou J, Tawk M, Tiziano FD, Veillet J, Bayes M, Nolet F, Garcia V, Servidei S, Bertini E, Castro-Ciner F, Renda Y, Carpentier S, Andrieu-Abadie N, Gut I, Levade T, Topaloglu H, Melki J: Spinal muscular atrophy associated with progressive myoclonic epilepsy is caused by mutations in ASAH1. Am J Hum Genet 2012, 91:5−14
9. Li G, Kidd J, Kaspar C, Dempsey S, Bhat OM, Camus S, Ritter JK, Gehr TWB, Gulbins E, Li PL: Podocytopathy and nephrotic syndrome in mice with podocyte-specific deletion of the Asah1 gene: role of ceramide accumulation in glomeruli. Am J Pathol 2020, 190:1211−1223
10. Bellomo G, Paciotti S, Gatticchi L, Parnetti L: The vicious cycle between α-synuclein aggregation and autophagic-lysosomal dysfunction. Mov Disord 2020, 35:34−44
11. Gatt S: Enzymic hydrolysis and synthesis of ceramides. J Biol Chem 1963, 238:3131−3133
12. Govindarajah N, Clifford R, Bowden D, Sutton PA, Parsons JL, Vimalachandran D: Sphingolipids and acid ceramidase as therapeutic targets in cancer therapy. Crit Rev Oncol Hematol 2019, 138:104−111
13. Ponnusamy S, Meyers-Needham S, Senkal CE, Saddoughi SA, Sentelle D, Selvam SP, Salas A, Ogretmen B: Sphingolipids and cancer: ceramide and sphingosine-1-phosphate in the regulation of cell death and drug resistance. Future Oncol 2010, 6:1603−1624
14. Ogretmen B: Sphingolipid metabolism in cancer signalling and therapy. Nat Rev Cancer 2018, 18:33−50
15. Roh JL, Park JY, Kim EH, Jang HJ: Targeting acid ceramidase sensitises head and neck cancer to cisplatin. Eur J Cancer 2016, 52:163−172
16. Lai M, Realini N, La Ferla M, Passalacqua I, Matteoli G, Ganesan A, Pistello M, Mazzanti CM, Piomelli D: Complete acid ceramidase ablation prevents cancer-initiating cell formation in melanoma cells. Sci Rep 2017, 7:7411
17. Hanker LC, Karn T, Holtrich U, Gätje R, Rody A, Heinrich T, Ruckhäberle E, Engels K: Acid ceramidase (AC)−a key enzyme of sphingolipid metabolism−correlates with better prognosis in epithelial ovarian cancer. Int J Gynecol Pathol 2013, 32:249−257
18. Yu FPS, Amintas S, Levade T, Medin JA: Acid ceramidase deficiency: farber disease and SMA-PME. Orphanet J Rare Dis 2018, 13:121
19. Chavez JA, Summers SAA: Ceramide-centric view of insulin resistance. Cell Metab 2012, 15:585−594
20. Ruvolo PP, Deng X, Ito T, Carr BK, May WS: Ceramide induces Bcl2 dephosphorylation via a mechanism involving mitochondrial PP2A. J Biol Chem 1999, 274:20296−20300
21. Weinberg JM: Lipotoxicity. Kidney Int 2006, 70:1560−1566
22. Guebre-Egziabher F, Alix PM, Koppe L, Pelletier CC, Kalbacher E, Fouque D, Soulage CO: Ectopic lipid accumulation: a potential cause for metabolic disturbances and a contributor to the alteration of kidney function. Biochimie 2013, 95:1971−1979
23. Choi SR, Lim JH, Kim MY, Kim EN, Kim Y, Choi BS, Kim YS, Kim HW, Lim KM, Kim MJ, Park CW: Adiponectin receptor agonist AdipoRon decreased ceramide, and lipotoxicity, and ameliorated diabetic nephropathy. Metabolism 2018, 85:348−360
24. Najafian B, Tøndel C, Svarstad E, Gubler MC, Oliveira JP, Mauer M: Accumulation of globotriaosylceramide in podocytes in fabry nephropathy is associated with progressive podocyte loss. J Am Soc Nephrol 2020, 31:865−875
25. Fornoni A, Sageshima J, Wei C, Merscher-Gomez S, Robier AP, Jauregui AN, Li J, Mattiazzi A, Ciancio G, Chen L, Zilleruelo G, Abitbol C, Chandar J, Seeherunvong W, Ricordi C, Ikehata M, Rastaldi MP, Reiser J, Burke GW: Rituximab targets podocytes in recurrent focal segmental glomerulosclerosis. Sci Transl Med 2011, 3:85ra46