CHAPTER 8

GLYCOSPHINGOLIPIDS AND KIDNEY DISEASE

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Abstract: Glycosphingolipids, derived from the addition of sugar-moieties to the sphingolipid ceramide, are highly abundant in the kidney. Glycosphingolipids are known to play an important role in organ function at least in part from inherited lipid storage diseases such as Anderson-Fabry disease (Fabry's disease; FD) that results from a mutation in alpha-galactosidase A (α-GLA or α-Gal A), the enzyme responsible for catalyzing the removal of terminal galactose residues from glycosphingolipids. The inactivation in α-GLA in FD results in the accumulation of glycosphingolipids, including globosides and lactosylceramides, which manifests as several common pathologies including end-stage kidney disease. More recently, glycosphingolipids and other sphingolipids have become increasingly recognized for their roles in a variety of other kidney diseases including polycystic kidney disease, acute kidney injury, glomerulonephritis, diabetic nephropathy and kidney cancer. This chapter reviews evidence supporting a mechanistic role for glycosphingolipids in kidney disease and discusses data implicating a role for these lipids in kidney disease resulting from metabolic syndrome. Importantly, inhibitors of glycosphingolipid synthesis are well tolerated in animal models as well as in humans. Thus, an increased understanding of the mechanisms by which altered renal glycosphingolipid metabolism leads to kidney disease has great therapeutic potential.

INTRODUCTION

Glycosphingolipids are a very heterogeneous class of lipids, varying dramatically in both the hydrophilic and hydrophobic region of the molecule. The hydrophilic portion of glycosphingolipids consists of a sugar headgroup and can contain neutral or charged groups. The hydrophobic portion can vary in the length of the *N*-linked fatty acyl chain and sphingoid base as well as the degree of unsaturation, hydroxylation and branching. Glycosphingolipids have numerous roles in regulating cellular processes,

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including cell proliferation, apoptosis, inflammation and cellular signaling. Dysregulation of glycosphingolipid metabolism leads to the accumulation of particular species of glycosphingolipids and induces several different pathologies and developmental abnormalities as evidenced from knockout studies in mice. Thus, it is not surprising that glycosphingolipids have been implicated in numerous diseases and that mutations in several enzymes involved in glycosphingolipid metabolism occur in a wide variety of human diseases. Glycosphingolipids are particularly abundant in the kidney and are thought to play an important role in kidney function. Indeed, altered glycosphingolipid metabolism occurs in a variety of kidney diseases. This chapter reviews current literature on the role of glycosphingolipids in kidney disease and discusses implications for their potential role in kidney pathologies associated with metabolic disease.

METABOLISM OF SPHINGOLIPIDS

Glycosphingolipids belong to the sphingolipid family, which all share a common sphingoid base backbone. Sphingolipid metabolism is a complex process involving many different sphingolipids and enzymes each with important and distinct cellular functions.[1] At the heart of sphingolipid metabolism is ceramide, which is comprised of a *N*-acylated (14-26 carbons) sphingosine (16 or 18 carbons). Ceramide can be generated by multiple pathways in cells, namely sphingomyelin hydrolysis, de novo synthesis, the salvage pathway, or breakdown of more complex sphingolipids (see Fig. 1). Sphingomyelinases (SMases) catalyze the hydrolysis of sphingomyelin (SM) to form ceramide and phosphorylcholine and are classified by their pH optima.[2,3] Of these, the acid and Mg^{+2}-dependent neutral sphingomyelinases have been implicated in stress-induced ceramide generation.[2,3]

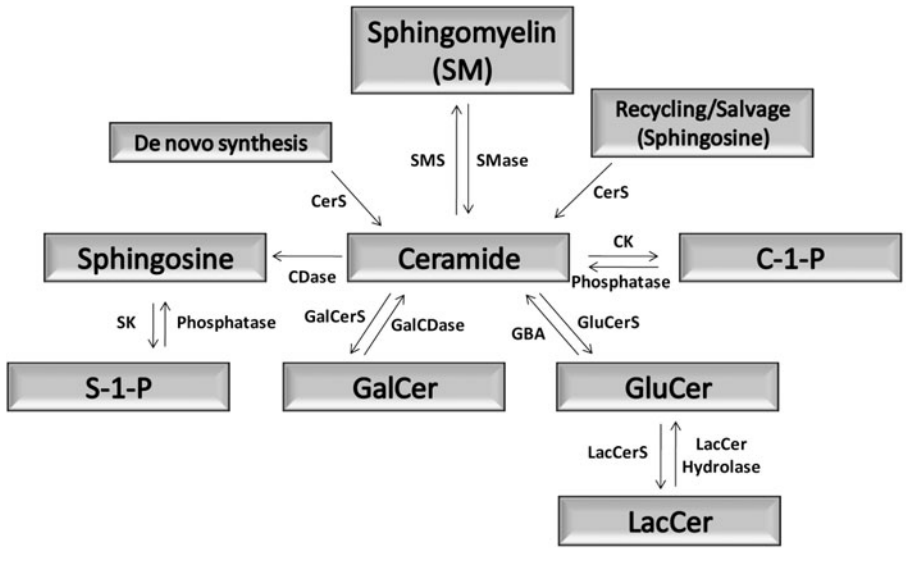

Figure 1. Skematic overview of sphingolipid metabolism.

De novo ceramide synthesis[4,5] occurs in the ER.[6] The enzyme serine palmitoyl transferase (SPT) catalyzes the first and rate limiting step in de novo synthesis, namely the condensation of serine and palmitoyl-CoA to form 3-ketosphinganine. The enzyme 3-ketosphinganine reductase catalyzes the reduction of 3-ketosphinganine to form sphinganine. Ceramide synthases catalyze the *N*-acylation of sphinganine to form dihydroceramide (DH). A desaturase catalyzes the conversion of dihydroceramide to ceramide through the insertion of a trans double bond at the 4-5 position of the sphingoid base backbone.

Once generated, ceramide's *N*-linked fatty acyl chain can be removed to generate sphingosine as catalyzed by ceramidases (CDase). Several CDases have been identified in mammals and are classified by their pH optima.[7] The sphingosine generated by the action of CDases can be used by ceramide synthases to regenerate ceramide in the recycling/salvage pathway. Thus, ceramide synthases are thought to occupy a central position in the sphingolipid metabolic pathway as they catalyze the formation of ceramide in two distinct pathways: de novo synthesis and the recycling/salvage pathway (see Fig. 1).

In mammals, there are six ceramide synthase isoforms (CerS1-6) that have preferences for fatty acyl CoAs of particular chain lengths[8-10] to form the corresponding ceramides. It is becoming increasingly recognized that specific CerS and their resulting ceramide products contribute differently to cellular processes.[11-23] Once generated, ceramides can serve as a metabolic precursor to complex sphingolipids, such as SM and glycosphingolipids. Of note, synthesis of complex sphingolipids can influence other lipid metabolic pathways; for example sphingomyelin synthase can catalyze the transfer of a phosphocholine headgroup from phosphatidylcholine to ceramide to generate SM and diacylglycerol in the Golgi apparatus. Ceramide can also be phosphorylated by ceramide kinase to generate ceramide-1-phosphate (C1P).

Ceramide is broken down when its *N*-linked fatty acyl chain is removed to liberate sphingosine (catalyzed by CDase), which in turn is phosphorylated to generate sphingosine-1-phosphate (S1P) as catalyzed by sphingosine kinases (SK). Two isoforms of SK have been cloned and characterized in mammals, SK1 and SK2. These enzymes are emerging as important and regulated enzymes that not only modulate the levels of S1P, but also those of sphingosine and ceramide.[24,25] S1P is broken down to yield hexadecenal and ethanolamine phosphate as catalyzed by sphingosine lyase. S1P is a pro-inflammatory, pro-proliferative and anti-apoptotic sphingolipid.[25] The dynamic balance between the levels of different sphingolipids is tightly regulated by the activities of the enzymes that catalyze their formation and breakdown. The relative cellular levels of different sphingolipids are proposed to influence cellular fate because of their opposing effects and their ability to be interconverted.

METABOLISM OF GLYCOSPHINGOLIPIDS

In general, glycosphingolipids are initially divided into one of two main classes based on the first sugar moiety linked to the ceramide backbone, namely galactose or glucose of galactosylceramide (GalCer) and glucosylceramide (GluCer), respectively. The GalCer is formed in the ER via the enzyme UDP-Gal:ceramide galactosyltransferase or GalCer synthase (CGalT) via the transfer of galactose from UDP-galactose to ceramide (see Fig. 1). GalCer is then transported to the Golgi where sulfatide or galactose groups can be added, processes which mainly occur in the kidney.

Glucosylceramides are generated from ceramides synthesized in the ER that are transported to the Golgi where UDP-Glc:ceramide glucosyltransferase (glucosylceramide synthase) catalyzes the addition of glucose onto ceramide from an activated nucleotide precursor (UDP-glucose). This reaction occurs on the cytosolic face of the Golgi membranes. In mammals, a galactose group is then added from UDP-galactose to generate lactosylceramide, a reaction catalyzed by lactosylceramide synthases on the luminal surface of the Golgi. In fact, all other stepwise additions to lactosylceramide occur in the Golgi lumen. The flipping of the GlcCer from the cytosolic to the luminal leaflet of the membrane for its metabolism is a highly energetically unfavorable event and is thought to be catalyzed by a flippase.[26] Additionally, it has been proposed that the protein glycosphingolipid transport protein FAPP2 may also be involved in this transbilayer movement of GlcCer.[27]

Several different sequential modifications to lactosylceramides can occur in the lumen of the Golgi to give rise to the more complex gangliosides, lactosylsulfatides, and globotriosylceramides.[28] Thus, LacCer is essential to the formation of complex glycosphingolipids. These complex glycosphingolipids arise from additions to either the 3-O- or the 4-O-position of lactosylceramide and involve combinations of alpha- or beta-linked groups, including glucose, galactose, N-acetylglucosamine, N-acetylgalactosamine and N-acetylneuraminic acid (sialic acid) (see Fig. 2). Gangliosides are a class of complex glycosphingolipids that arise from the addition of sialic acid residues to lactosylceramide. Their names are abbreviations assigned to them according to the number of sialic acid residues and the order in which they migrate in chromatography. (For a review of glycosphingolipid metabolism, for description of the root names, or glycosphingolipid structures see refs. 29-33). Gangliosides are highly abundant in the kidney and are thought to play an important role in numerous kidney functions as described below.

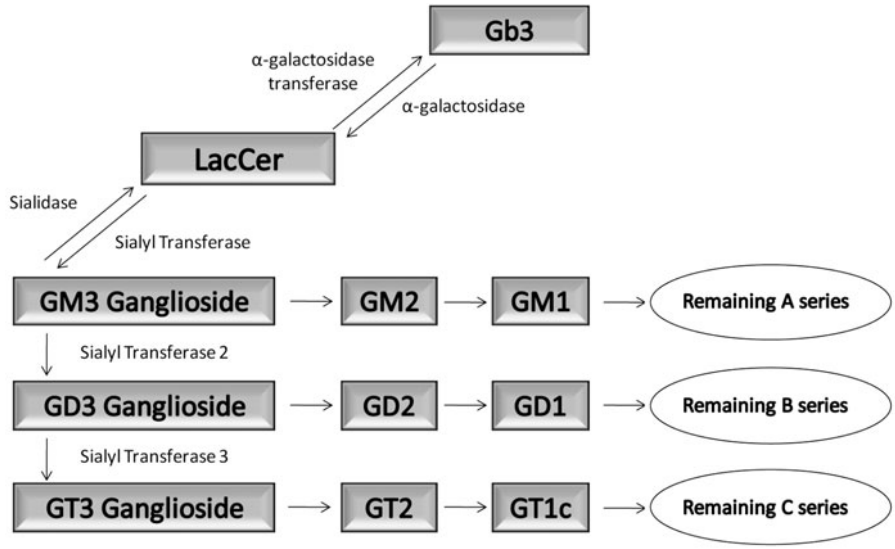

Figure 2. Skematic overview depicting of the major pathways of glycosphingolipid metabolism.

Glycosphingolipids can also be formed by degradation of more complex glycosphingolipids, which occurs mainly in the lysosome, but is known to occur at the plasma membrane and mitochondrion as well.[33-41] Indeed, several lysosomal storage diseases result from mutations in enzymes responsible for the degradation of glycosphingolipids.[32,42-49] Defective degradation of glycosphingolipids can result in serious pathologies as is described below for Fabry's kidney disease.

GLYCOSPHINGOLIPID SUBCELLULAR LOCALIZATION AND TRANSPORT

Glycosphingolipids are found in several cellular locations. On the outside of the plasma membrane, glycosphingolipids are a minor component except for the apical plasma membrane of epithelial cells in the urinary and intestinal tracts where they are highly enriched in the apical plasma membrane, representing 30-40 mole percent of the total lipids.[50-53] The myelin is enriched in galactosylceramide derived glycosphingolipids and the neuronal plasma membranes have particularly high levels of gangliosides. Intracellularly, glycosphingolipids are also found in the exocytic and endocytic pathways. Mitochondria and mitochondrial associated ER derived membranes (MAMs) have been reported to contain particular glycosphingolipids, namely GD1 and GD3 gangliosides and particular enzymes of glycosphingolipid metabolism have been localized to mitochondria and/or MAMs, including neuraminidase isoform 4 which is a sialidase and can act on both gangliosides and glycoproteins.[40,41] Enzymes that breakdown glycosphingolipids are also localized to lysosomes with inactivating mutations leading to the accumulation of particular species of glycosphingolipids as is the case with Fabry's disease and numerous others. In addition, there are also a few enzymes responsible for breakdown of glycosphingolipids that localize to the cytosol, mitochondria and the plasma membrane as is the case for sialidase isoforms 2, 3 and 4.[36,38-41,54-56] As the majority of glycosphingolipids are synthesized in the Golgi, but are localized in other subcellular locations, a system for their transport must exist.

The main system for transport of glycosphingolipids is vesicular along the exocytic and endocytic recycling pathways, which is consistent with their synthesis on the luminal side of the Golgi and enrichment in luminal surface of the endocytic vesicles and the outer surface of the plasma membrane as described in detail elsewhere.[33,57-59] It is interesting that despite the fact that vesicular transport is bidirectional, glycosphingolipids are preferentially maintained in certain subcellular compartments over others. Data in the literature suggest that it is a highly regulated process and is cell-type specific.[33,57-59] Lipid rafts and caveolin are thought to play an important role in the preferential sorting of glycosphingolipids.[58,60-62] In addition to vesicular transport, there is evidence to suggest that glucosylceramide transport to the plasma membrane occurs independently of the Golgi pathway and a glycosphingolipid transport protein has been identified.[63,64]

FUNCTIONS OF GLYCOSPHINGOLIPIDS

Glycosphingolipids regulate several different cellular processes, including cell proliferation, cell signaling, apoptosis and cell recognition. (Only an overview of the function of glycosphingolipids is given below and is not the purpose of this chapter.

For additional information the reader is referred to[28,65-74] and additional references in the section to follow). The study of roles of glycosphingolipids in cell proliferation has been facilitated through studies in mouse models.[32] Glycosphingolipids, including lactosylceramide and galactosylceramide are elevated in several types of tumors.[28,66,75-91] It has been proposed that their positive regulation of cell proliferation promotes tumor formation and growth.[28,69,92-99] Increased production of certain glycosphingolipids inhibits apoptotic mechanisms of the cell and potentially causes cancer.[100] Indeed, increased conversion of the pro-apoptotic sphingolipid ceramide to glucosylceramide occurs in a variety of cancer cells and is thought to play a role in resistance of cancer cells to radiation and chemotherapy.[85,101,102] Alternatively, other glycosphingolipids such as GD3 ganglioside have been shown to play a role in apoptosis at least in part through induction of cytochrome c release from mitochondria.[100,103-117] It is currently unclear how GD3 ganglioside is transported to mitochondria to exert its pro-apoptotic role. However, a mitochondrial role for GD3 ganglioside is supported at least in part by the fact that the sialidase isoform NEU4 localizes to mitochondrial outer membranes and that GD3 ganglioside is one of its known substrates.[41,106]

Glycosphingolipids, especially GM3, are especially important in signal transduction pathways through their carbohydrate interactions.[118] Lipid rafts in the plasma membranes of cells are lipid microdomains thought to be enriched in glycosphingolipids, sphingomyelin and cholesterol and have been proposed to play an important role in cell signaling and cell recognition.[65] Glycosphingolipid containing lipid rafts at the plasma membrane are thought to be important in organizing membrane receptors, helping to transmit various aspects of signal transduction pathways[65] and in cellular surface recognition.[119-127] At the plasma membrane, gangliosides faciliate cell-cell adhesion and are important in recognition of growth factors and immune responses.[120-127]

Gangliosides activate macrophages, inducing the production of proinflammatory cytokines.[32,128] The role of glycosphingolipids in the inflammatory response has been well studied in neuroinflammatory disease and glycosphingolipids like LacCer are throught to play an important role.[128] Glycosphingolipid accumulation in lysosomal storage diseases activates an inflammatory response at least in part due to dysfunctional trafficking.[129] The use of anti-inflammatory agents in mutant mouse models of glycosphingolipid storage diseases reduces disease progression, suggesting that glycospingolipids act in conjunction with other pro-inflammatory factors to mediate inflammation.[32] TNF-α induced ceramide has been implicated in the activation of transcription factors that encode various cytokines and chemokines.[130] Indeed, TNF-α has also been implicated in LacCer metabolism through its activation of nSMase and production of ceramide.[128,130] β-glucosidase 1 (GBA) downregulation increases production of the cytokine IL-6, which plays an important role in inflammation.[131] Pharmacological inhibition of glycosphingolipid synthesis in leptin-deficient Ob mice reduced levels of inflammatory markers in adipose tissue and improved glycemic control, further implicating the pro-inflammatory function of glycosphingolipids as the mechanism by which they mediate disease.[132]

Malfunctions in the concentrations and arrangement of glycosphingolipids can have devastating effects on many organ systems.[32] Glycosphingolipids are found in many types of cells, but are particularly abundant in the myelin sheath and epithelial cells of the renal tubules.[33] In the kidney, defects in glycosphingolipid metabolism that either result in their accumulation or enhanced degradation occurs in a variety of kidney diseases as described in the sections to follow.

FABRY'S DISEASE

Fabry's disease is an example of a lipid storage disease that in the last forty years has progressed from beginning to understand its cause to the development of effective treatments; much of this progress has been driven by breakthroughs in basic scientific research.[133] Fabry's disease is a rare X-linked lysosomal storage disease that results from mutations in the gene that encodes the lysosomal enzyme alpha-galactosidase A (α-GLA or α-Gal A). Over 150 different mutations in the gene that encodes for α-GLA have been documented to lead to the defective or reduced enzyme activity in Fabry's disease and these mutations have been described in all 7 exons of the gene.[134] The disease is estimated to occur in 1 out of every 117,000 male live births and the estimated survival in males is approximately 50 years without the use of recently available treatments.[135] Hemizygous males frequently suffer from severe renal disease and middle age onset of end stage renal failure (ESRF). Heterozygous females normally do not present with proteinuria or ESRF.[129,136,137]

α-GLA is responsible for catalyzing the hydrolysis of terminal galactosyl species from globotriaosylceramide (galactosyl-α-galactosyl-β-glucosylceramide; Gb3) as well as other glycolipids and glycoproteins (see Fig. 2). The glycosphingolipid Gb3 is one of the main glycolipid species that accumulates within the lysosomes of cells in Fabry's disease patients, resulting in the characteristic lamellar lysosomal membrane structures in cells; the most profound accumulations of Gb3 in Fabry's disease occur in the heart and kidney as well as endothelial cells throughout the vasculature.[129] α-GLA expression has been documented in all types of renal tubular and interstitial cells[138] in normal kidneys. In Fabry's disease Gb3 buildup occurs in several areas within the kidney, including glomerular cells (podocytes, mesangial and endothelial cells) and epithelial cells of the Loop of Henle and in the distal tubule, impairing the ability of the kidneys to form concentrated urine.[134] Normally, Fabry's disease patients present with proteinuria, along with tubular malfunctions such as vasopressin-resistant nephrogenic diabetes insipidus and distal tubule acidosis.[136] Advanced Fabry's disease normally leads to ESRF, but onset of ESRF depends on the type of mutation and degree of α-GLA activity present. Management of symptoms associated with kidney malfunction is required along with dialysis and renal transplantation in patients with ESRF.[134] Treatment for Fabry's through enzyme replacement with α-GLA has shown an increase in α-GLA activity, but the damage already sustained to the kidneys is often irreversible.[129] Thus, early diagnosis and intervention is key to the most effective treatment.

Although no definite mechanism has been proposed, there are several hypotheses explaining the progression of Fabry's disease to complete renal failure. Many environmental factors could contribute, but it is thought that ESRF is brought on by vascular changes inside the kidney that eventually lead to ischaemic nephropathy.[136] Other hypotheses include the accumulation of neutral glycosphingolipids that can have been shown to alter charge selective filtration as well as elevated inflammation within the kidney.[32,129,139]

POLYCYSTIC KIDNEY DISEASE

Polycystic kidney disease (PKD) represents a wide variety of renal disorders that are characterized by cystic growth in the kidneys, eventually leading to kidney failure. PKD is a common genetic disorder that affects over 500,000 Americans, with 7,000 new cases every year.[99] The major form of PKD is the result of an autosomal dominant mutation in

polycystin-1 and -2 and has been mapped to human chromosome 6.[99] Recessive forms of the disease result in childhood onset and early end-stage renal failure (ESRF).[140] Patients normally present with enlarged kidneys due to the presence of cysts in the collecting duct of the nephron. Cysts grow in size and number with increasing age. Although there are many forms of PKD, most are caused by uncontrolled tubular epithelial cell proliferation, altered regulation of apoptosis and the activation of growth factors.[141] Glycosphingolipids such as glucosylceramide (GluCer), lactosylceramide (LacCer) and GM3 have shown to regulate these processes.

The role of glycosphingolipid metabolism in PKD has been studied in several mouse models of PKD. The renal levels of GM3 and GluCer were found to be higher in PKD mouse models than in the corresponding control mice, suggesting that these glycosphingolipids might play a role in PKD. Renal glycosphingolipids are elevated in both the recessive and dominant forms of PKD have accumulations of renal glycosphingolipids. The PKD mouse models jck, Pkd1 cKO and pcy all show elevated levels of GM3, GluCer, with slightly increased, but not statistically significant, increases in ceramide. These sphingolipid profile changes are consistent with those present in human PKD kidneys.[141]

The inhibition of glycosphingolipid synthesis and the subsequent reduction in their concentrations has shown promise for translating to new treatments for PKD. The GluCer inhibitor Genz-123346 inhibits the glucosylceramide synthase and depletes GluCer without inducing accumulations in ceramide.[141] Studies have shown that administration of Genz-123346 to mouse models of PKD decreased renal levels of GluCer and GM3.[141] Importantly, Genz-123346 not only reduced cyst volume and fibrosis, but also delayed the progression/onset of PKD in mouse models.[141]

The role of glycosphingolipids in the regulation of cell cycle as well as activation of growth factors is thought to be mechanisms by which they mediate PKD development. The improvement in PKD with Genz-123346 is thought to be due to decreased cell proliferation through regulation of cell cycle progression as its use on kidney cells grown in culture delayed progression of the cell cycle at G2/M.[141] The activation of growth factors, particularly epidermal growth factors (EGF), through the phosphorylation of mitogen-activated protein kinases (MAPK) has been linked to the formation of cysts along with increased inflammation due to the upregulated activity of cyclic-AMP.[99] Importantly, in vitro lactosylceramide addition to human kidney proximal tubule cells induces phosphorylation of MAPK.[99] The recent development of novel inhibitors of glucosylceramide synthase that are well tolerated in mice as well as in humans shows much promise for treating PKD in humans.

KIDNEY CANCER

Several types of glycosphingolipids are elevated in a variety of tumors. In renal cancer, glycosphingolipids reported to be elevated include GM1, GM2, GD2, GD3 and NeuGc-GM3.[142-145] Other glycosphingolipids have been found at much lower levels in kidney cancers. For example, the glycosphingolipid disialylgalactosylgloboside and the sialyltransferase ST6GalNAc VI responsible for its synthesis were found to be downregulated in renal cancer cell lines and cancer tissues.[146] Indeed, human renal cell carcinomas (RCC) are characterized by significant changes in ganglioside composition, which is supported by documented changes in expression of enzymes involved in their metabolism in RCC.[87,147,148] For example, the sialidase NEU3 specifically degrades

gangliosides and is markedly upregulated in many renal carcinomas.[87] In addition, the glycosphingolipid GalNAc disialosyl lactotetraosylceramide is abundant in metastatic renal cell carcinomas in humans.[149] Thus, altered glycosphingolipid metabolism may be key to the growth and metastasis of renal tumors.

Various glycosphingolipids have been implicated in cellular proliferation as discussed above and this may be one mechanism by which they play a role in renal cancers. In addition to regulating cell proliferation, certain gangliosides have shown to inhibit the immune response mounted by the body against tumor cells. Gangliosides such as GM2, GD2 and GD3 accumulate in renal tumors and are shed from these tumors where they are taken up by activated T cells, inducing their apoptosis and causing immune dysfunction.[142,144,150] Understanding how glycosphingolipid metabolism is altered in renal cancers and the mechanism by which glycosphingolipids contribute to the formation and metastasis of renal tumors will allow this pathway to be targeted for the development of novel therapeutics. Indeed, inhibitors of glycosphingolipid synthesis are well tolerated in rodents as well as humans and could potentially be useful in the treatment of renal cell carcinoma.

GLOMERULONEPHRITIS

Glomerulonephritis encompasses several disorders grouped according to varying symptoms such as proteinuria, renal hypertrophy and inflammation with some variations culminating in end-stage renal disease.[151,152] Although no single mechanism has been accepted, recent studies indicate that the loss of a negative charge on proteins, glycolipids and carbohydrates in the podocytes is at least in part responsible for the decreased filtration of the glomerular capillaries.[153-155] Sialic acid is located on the outside of the podocyte and it is responsible for the filtration due to the charge repulsion of adjacent negatively charged sialic acids. This repulsion is at least in part responsible for the creation of filtration gaps.[151] The loss of sialic acid on the surface of the podocytes has been linked to proteinuria, a major indicator of human glomerular disease.[151] Sialic acid residues are cleaved by sialidases (neuraminidases) that are present in the plasma membrane, cytosol, mitochondria and lysosomes.[155] Indeed, removal of negatively charged sialic acid residues from the apical plasma membrane of podocytes via in vivo treatment with bacterial sialidase is sufficient to induce foot process effacement and proteinuria.[156-159] Importantly, the kidneys of mice carrying a mutated form of a key enzyme involved in the production of sialic acid have podocyte effacement and splitting of the glomerular basement membrane resulting in severe proteinuria that is partially rescued with dietary supplementation,[160,161] further illustrating the importance of sialic acid residues in podocyte function.

Sialidases do indeed desialylate the gangliosides GM3, GM2 and GM1.[155] The plasma membrane sialidase NEU3, acts exclusively on gangliosides and in renal cancers has been shown to be upregulated.[162] This upregulation leads to an accumulation of lactosylceramide with a concomitant decrease in GM3, influencing the ratio between negatively charged and neutral glycosphingolipids which alters the balance between cell proliferation and cell death.[162] Likewise, in puromycin induced mouse models of minimal change nephropathy, a disappearance of GD3 was noted at day 10.[163] Around day 30, the gangliosides were measured again and found to be close to those of the control mice with disease symptoms subsiding.[163] These data suggest that the loss of renal gangliosides plays a major role in minimal change nephropathy.[163]

Sialidases have been implicated in other glomerular diseases and patterns of glomerular injury, including lupus nephritis, primary and secondary focal segmental glomerulosclerosis (FSGS) and membranous glomerulopathy. Lupus nephritis is an autoimmune systemic disease which has a variable histology including membranous injury and/or extracapillary, mesangiocapillary, or mesangial proliferation and characterized by decreased renal filtration, proteinuria and inflammation. The decreased renal filtration is due to the destruction of the foot processes of the podocytes,[164] which has been proposed to be at least in part caused by defective removal of sialic acid residues from glycoproteins and glycolipids via increased activity of sialidases, resulting in the closure of filtration slits.[151] Our own unpublished data indicates that the sialidase Neu1 is upregulated in a mouse model of lupus nephritis and that the substrate for Neu1 is not a glycoprotein, but rather the ganglioside GM3 as there are concomitant accumulations in the neutral glycosphingolipid products lactosylceramide and glucosylceramide. It is possible that the immune cells within the kidney are the source of changes in glycosphingolipid metabolism as GM1 gangliosides levels are altered in the peripheral CD4+ T cells of lupus patients.[165] As lupus nephritis is an autoimmune disorder, it is not surprising we detect altered glycosphingolipid metabolism as gangliosides have long been known to play a role in a variety of autoimmune diseases.[166-172] In glomerular diseases, studies have focused on identifying changes in the patterns of the glycol-epitopes on glycoproteins in the glomerular basement membrane with little success. The decrease in negative charges was once thought to be due to loss of heparan sulfate, but this has been challenged by studies indicating that its removal from the glomerular basement membrane was insufficient to induce proteinuria.[173,174] In addition, studies examining changes in the patterns of sialoproteins have not identified a particular protein target with decreased sialysation despite evidence of increased sialic acid in the urine.[153,175-177] Even though the patient numbers were very low, one study showed an elevated level of the sialidase Neu1 of membranous glomerulopathy with elevated urinary sialic acid levels, but not changes in the glycoprotein dystroglycan.[153] Thus, it is possible that the negatively charged sialic acid residues are lost not from glycoproteins such as dystroglycan, but rather from gangliosides.

DIABETIC NEPHROPATHY AND METABOLIC SYNDROME

In STZ-induced diabetic rats, the levels of kidney glucosylceramides are significantly elevated without changes in the activity of glucosylceramide synthase.[178] However, kidney levels of glucose and UDP-glucose (a substrate for the formation of glucosylceramides) are also significantly elevated in the kidneys of STZ-induced diabetic rats.[178] Importantly, Zador et al 1993 found that the level of UDP-glucose present in the kidney under normal conditions was below the Km for glucosylceramide synthase, indicating that it is a rate limiting substrate in the synthesis of glycosphingolipids.[178] Glycosphingolipid metabolic enzymes have been shown to be regulated in vivo by elevated glucose utilization.[179] When liver glucose utilization was elevated via the overexpression of glucokinase (hexokinase IV) in the livers of STZ-induced Type I diabetic rats, there was a 6-fold upregulation in the levels of lactosylceramide synthase mRNA.[179] Alternatively, glycosphingolipids may regulate directly or indirectly glucose utilization through regulation of insulin signaling.[180,181] GM3 is thought to play a role in insulin resistance as mutant mice deficient in this ganglioside have increased insulin sensitivity.[182] In addition, pharmacological inhibition of glucosylceramide synthase in ob/ob mice that are insulin resistant was sufficient to improve glucose tolerance.[180] Likewise, inhibition of glucosylceramide synthase with two different glucosylceramide

synthase inhibitors improved glucose tolerance and insulin sensitivity in diet-induced as well as STZ-induced diabetic mice.[180,181] Reduced glycosphingolipid levels in cells induces GLUT4 translocation to the plasma membrane via enhanced formation of GLUT4 storage vesicles.[183] This recent data from the Pagano laboratory provides mechanistic insight for in vivo studies utilizing glucosylceramide synthase inhibitors to improve glycemic control and insulin sensitivity in rodent models of diabetes.[183]

Chronic hyperglycemia and insulin resistance dramatically alter kidney function and lead to diabetic nephropathy. Indeed, complex glycosphingolipids have been implicated in diabetic nephropathy where changes in glomerular sialic acids and/or sialidase activity correlate with onset of proteinuria.[184-186] In addition, studies indicate a role for ceramide in renal injury resulting from high fat diet induced nephropathy.[187] No measurements of glycosphingolipids were reported in these studies, but given that ceramides are required for the formation of glycosphingolipids, it is possible that they also are also elevated and play a role.[187] A link between glucose metabolism, insulin resistance and glycosphingolipid metabolism is clear from numerous reports in the literature,[180,181,183,188-190] suggesting a role for glycosphingolipids in diabetic nephropathy and kidney disease resulting from metabolic syndrome.

CONCLUSION

Data in the literature clearly indicate a role for glycosphingolipids in a variety of kidney diseases, including Fabry's kidney disease, polycystic disease, kidney cancers, several types of glomerulonephritis and diabetic nephropathy. Data strongly suggest a role for glycosphingolipids in kidney disease resulting from metabolic syndrome and future studies are aimed at uncovering these specific roles. Data with novel inhibitors of glucosylceramide synthase that deplete kidney glycosphingolipids without elevating ceramides show promise, not only for the translation to treatment of polycystic kidney disease in humans, but also the other kidney diseases mentioned above.

Kidney glycosphingolipids encompass a complex and highly diverse family of lipids with diverse cellular roles. Thus, the identification of the specific glycosphingolipid species involved in specific kidney pathologies is essential. The recent development of novel mass spectrometry techniques that allow for the quantification of complex glycosphingolipids will help with this challenging and essential endeavor. Inhibitors of glucosylceramide synthase deplete a plethora of different glycosphingolipids and development of additional inhibitors that target specific points in the glycosphingolipid metabolic pathway is essential. In addition, deciphering the mechanism by which the accumulation of specific glycosphingolipid species leads to specific kidney pathologies will allow for the identification of novel therapeutic targets for the development of drugs aimed at interfering with their actions in addition to their metabolism.

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