The proteinuria—hypertriglyceridemia connection as a basis for novel therapeutics for nephrotic syndrome

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The development of new and specific treatment options for kidney disease in general and glomerular diseases in specific has lagged behind other fields like heart disease and cancer. As a result, nephrologists have had to test and adapt therapeutics developed for other indications to treat glomerular diseases. One of the major factors contributing to this inertia has been the poor understanding of disease mechanisms. One way to elucidate these disease mechanisms is to study the association between the cardinal manifestations of glomerular diseases. Because many of these patients develop nephrotic syndrome, understanding the relationship of proteinuria, the primary driver in this syndrome, with hypoalbuminemia, hypercholesterolemia, hypertriglyceridemia, edema, and lipiduria could provide valuable insight. The recent unraveling of the relationship between proteinuria and hypertriglyceridemia mediated by free fatty acids, albumin, and the secreted glycoprotein angiopoietin-like 4 (Angptl4) offers a unique opportunity to develop novel therapeutics for glomerular diseases. In this review, the therapeutic potential of mutant forms of Angptl4 in reducing proteinuria and, as a consequence, alleviating the other manifestations of nephrotic syndrome is discussed. (Translational Research 2015;165:499–504)

Abbreviations: Angptl4 = Angiopoietin-like 4; CG = collapsing glomerulopathy; FFA = free fatty acid; FSGS = focal and segmental glomerulosclerosis; GBM = glomerular basement membrane; HIV = human immunodeficiency virus; LPL = lipoprotein lipase; MCD = minimal change disease; MN = membranous nephropathy; PPAR = peroxisome proliferator-activated receptor; RAS = renin angiotensin system

INTRODUCTION

Nephrotic syndrome is characterized by the presence of proteinuria in excess of 3.5 g per 24 hours, hypoalbuminemia, and variable amounts of hyperlipidemia (hypertriglyceridemia and hypercholesterolemia), lipiduria, and edema.[1] Patients with primary glomerular diseases (eg, minimal change disease [MCD], focal and segmental glomerulosclerosis [FSGS], membranous nephropathy) and systemic disorders (eg, diabetes mellitus, systemic lupus erythematosus, and amyloidosis) can present with nephrotic syndrome. Substantial research effort has been committed toward understanding the pathogenesis of each of the individual components. For example, several new proteins expressed in podocytes have been implicated in the pathogenesis of proteinuria,[2-6] and at least one novel therapeutic approach is being developed based on this knowledge.[1,6] We now understand how salt retention

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through different tubular segments contributes toward edema,[7] and this knowledge forms the basis of diuretic therapy, which is the mainstay of the treatment of edema. Changes in cholesterol uptake and the cholesterol biosynthetic pathway in hepatocytes have shed some light toward the development of hypercholesterolemia.[8] However, although nephrotic syndrome (including the precursor term "nephrosis") was recognized over a century ago, a molecular relationship between some of these has only very recently started becoming clear.

THE MOLECULAR LINK BETWEEN PROTEINURIA AND HYPERTRIGLYCERIDEMIA

A study published recently[9] established the first molecular link between proteinuria and the hypertriglyceridemia component of hyperlipidemia. Although investigating elevated circulating levels of the secreted glycoprotein angiopoietin-like 4 (Angptl4) in human MCD, FSGS, membranous nephropathy, non-human immunodeficiency virus (HIV) collapsing glomerulopathy, and in corresponding animal models, it was determined that levels rise after, but not before, the development of moderate to severe proteinuria. Elevated plasma levels of Angptl4, a previously known inhibitor of lipoprotein lipase (LPL, an enzyme that catalyzes conversion of triglycerides to monoglycerides and free fatty acids [FFAs]), coincide with the development of hypertriglyceridemia and reduced lipoprotein lipase activity. In addition, Angptl4 is essential for the development of hypertriglyceridemia in nephrotic syndrome, because plasma triglyceride levels do not increase in nephrotic mice that lack Angptl4. The bulk of this circulating neutral or near neutral isoelectric point form of Angptl4 originates from skeletal muscle, heart, adipose tissue, and additionally from podocytes in MCD. Circulating Angptl4 differs significantly from a podocyte-secreted hyposialylated, high isoelectric point form that causes the cardinal manifestations of human MCD, and does not appear in the circulation.

Using transgenic rats, knockout mice, and recombinant rat and human Angptl4, it was determined that circulating Angptl4 reduces proteinuria in nephrotic rodents by binding to a protein present in glomerular endothelial cells at their interface with the glomerular basement membrane, the $\alpha v\beta 5$ integrin. Using knowledge from previous human genetic studies,[10] distinct mutant forms of human Angptl4 were developed. These Angptl4 mutants reduce proteinuria in nephrotic rodents without significantly elevating plasma triglyceride levels. By contrast, recombinant wild-type human Angptl4 reduces proteinuria and elevates plasma triglyceride levels. These studies firmly establish that circulating Angptl4 reduces proteinuria, at the same time also inducing hypertriglyceridemia in nephrotic syndrome (Fig 1), and that these effects involve different sites in this protein.

NEGATIVE FEEDBACK LOOPS IN NEPHROTIC SYNDROME

The development of novel therapeutics using recombinant Angptl4 requires a broad understanding of the pathways involved. Angptl4 is a known peroxisome proliferator-activated receptor (PPAR)α[11] and PPARγ[12] target gene, and FFAs induce upregulation of Angptl4 expression in skeletal muscle and heart via a PPAR-dependent mechanism.[13-15] Circulating FFAs are noncovalently bound to plasma albumin. Patients with nephrotic syndrome lose albumin with low FFA content in the urine and retain albumin with higher FFA content in the circulation.[16] Our studies in patients with nephrotic syndrome because of MCD and FSGS also noted the FFA-to-albumin ratio to be significantly lower in the urine compared with plasma. Longitudinal studies in Buffalo Mna rats, a model of FSGS, revealed the presence of relatively low FFA containing urine albumin even during mild proteinuria, which eventually leads to a significant increase in the ratio of plasma FFA to albumin when proteinuria becomes moderate to severe (ie, "nephrotic range"). Higher albumin-bound FFA and the development of hypoalbuminemia both contribute toward the elevated FFA-to-albumin ratio that promotes FFA uptake and induces Angptl4 upregulation most likely via a PPAR-dependent mechanism. Increased PPAR expression is noted in peripheral organs (heart, skeletal muscle, adipose tissue, and liver) of nephrotic rats coinciding with Angptl4 upregulation. Finally, raising the ratio of plasma FFA to albumin in nephrotic Buffalo Mna rats by administering oleic acid, a monounsaturated $\omega 9$ fatty acid present naturally in animal and vegetable fats, or Intralipid (Fresenius Kabi AB, Uppsala, Sweden), a commercially available lipid supplement, increases plasma Angptl4 levels and significantly reduces proteinuria.

These studies demonstrate the presence of 2 negative feedback loops in nephrotic syndrome, illustrated in Fig 2. The first loop is a systemic loop that starts with proteinuria and ends with the reduction of proteinuria and includes urinary loss of albumin with low FFA content, elevated ratio of plasma FFA to albumin, upregulation of Angptl4 expression and secretion from peripheral organs, and binding of circulating Angptl4 to the glomerular endothelium. The second loop is local and restricted to skeletal muscle, heart, and adipose tissue, which express both Angptl4 and LPL. This loop starts with increased entry of FFA into these organs and ends with reduced entry of FFA. It includes the high