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NEPHROTIC SYNDROME

Negative feedback loop reveals novel potential therapy

Nephrotic syndrome has been recognized in the literature for over 90 years. However, the molecular background behind the clinical definition of nephrotic syndrome has not been established. Now, using data from patients and animal models, Sumant Chugh and his team have identified a negative feedback loop that should have important implications in terms of understanding and treating this disease.

Why patients with proteinuria have the symptoms of nephrotic syndrome, including hyperlipidaemia, only when proteinuria levels exceed 3.5 g per day has not been clear. That is, the link between proteinuria and the increase in circulating lipids was not known. Chugh and his team have now shown that the circulating angiopoietin-like 4 (Angptl4) protein is a link between these two systems.

This work has been a long time in development. Chugh explains: "in discovery phase studies back in 2002, we identified 40 genes of interest in rats with proteinuria. *ANGPTL4* was upregulated 70-fold in the glomeruli of these rats, and it causes hypertriglyceridaemia." So, the researchers had a strong indication that here was a gene that linked proteinuria with the hypertriglyceridaemia component of hyperlipidaemia.

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In 20 patients with nephrotic syndrome due to non-HIV collapsing glomerulopathy, six patients with membranous nephropathy, 10 patients with minimal change disease and 28 patients with focal and segmental glomerulosclerosis, a significant increase in plasma ANGPLT4 level was seen, compared with healthy controls. This finding implied that the genetic results are likely to be of direct relevance to patients.

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Therefore, Chugh and his team used nine different animal models of various nephrotic syndromes to pin down the interaction between Angptl4, proteinuria and hypertriglyceridaemia.

In these rat models of nephrotic syndromes, circulating Angptl4 was excreted by extrarenal organs—in particular the heart, skeletal muscle, adipose tissue and the liver—in response to an elevated plasma ratio of fatty free acids (FFAs) to albumin, but only when proteinuria had reached levels equivalent to a clinical definition of nephrotic syndrome in humans. "This is the first time that we understand what is behind the clinical range for proteinuria in nephrotic syndrome," Chugh continues, "this is the peripheral organs responding to proteinuria."

In a negative feedback loop, circulating Angptl4 then interacts with glomerular endothelial $\alpha_v\beta_5$ integrin to reduce proteinuria. At the same time, Angptl4's extrarenal interactions reduce tissue uptake of FFAs by skeletal muscle, heart and adipose tissue, resulting in hypertriglyceridaemia.

The researchers went back to the patient data to see if there might be therapeutic potential in these findings. There is a known variant in *ANGPLT4* at amino acid 40 that is important for inhibiting lipoprotein lipase activity, and amino acids 161–164 are involved in the cleavage of the full length protein. Mutations in both of these regions were introduced into the human gene, and recombinant human protein was produced using the mutated constructs.

Chugh has described these mutated human proteins as "the first ever biological agent developed specifically for nephrotic syndrome," and the results in rats support this claim. After intravenous injection of this recombinant protein into rat models of nephrotic syndrome, a significant reduction in proteinuria was observed compared to baseline. Although no dose-response assessments have been completed, the data indicate that this treatment caused a reduction in proteinuria that lasted for at least 2 weeks.

As this is an exogenous, mutated protein, it is proposed that it can interfere with the delicate negative feedback loop that controls the systemic response to proteinura in humans. This interference is achieved in a way that was not possible using other agents in the pathway, such as FFA. In addition, because it forms large oligomers and binds to HDL cholesterol particles, it is anticipated by the authors that this will be important in terms of the pharmacokinetics of the agent.

The next step for Chugh and his team is the high-throughput expression of the mutated proteins, which can then be used to conduct dose-response and preclinical toxicity studies in rats. The ultimate intention is the translation of this therapy into patients, and the fact that it is a human protein should assist with this endeavour.

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