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CIN85: Implications for the Development of Proteinuria in Diabetic Nephropathy

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COMMENTARY

Diabetic nephropathy (DN) is the most common cause of end-stage renal disease worldwide, and the therapy option is far from a solution. The earliest morphological change is glomerular basement membrane thickening, followed by mesangial expansion, predominantly because of an increase in mesangial matrix. The clinical manifestations of DN, such as microalbuminuria or proteinuria, are strongly related to these structural changes (1). However, the onset of albuminuria is also associated with podocytopathies in which several important podocyte slit diaphragm–associated proteins are involved (2). Podocyte slit diaphragm plays a pivotal role in maintaining the size-selective barrier demonstrated by the analysis of congenital nephrotic syndrome (3,4). Slit diaphragm–associated proteins, such as nephrin, CD2-associated protein (CD2AP), and podocin, have been investigated to clarify the phenotypical modification of podocytes in genetic disease (5). However, the complex regulation of dynamic functional interaction of these molecules, especially in DN, has been poorly understood.

CIN85 was independently identified as a Cbl-interacting protein of 85 kDa (6), Ruk (regulator of ubiquitous kinase) (7), SETA (SH3 domain-containing gene expressed in tumorigenic astrocytes) (8), and SH3KBP1 (SH3 domain-containing kinase binding protein 1) (9). Since its identification as a Cbl-interacting protein, CIN85 has been found to interact with many molecular partners through its SH3 domains, a proline-rich region, and a coiled-coil domain (Fig. 1). The identification of the particular "PxxPR" proline motif recognized by CIN85's SH3 domains has led to the discovery of numerous CIN85-interacting proteins, many of which are involved in the formation and trafficking of endocytic vesicles, an essential mechanism for the downmodulation of receptor tyrosine kinases (RTKs), as well as regulation of cell cytoskeleton and phospholipid metabolism (10,11). CIN85 and its paralogue CD2AP can form heterotypic complexes, which bind to F-actin and modulate dynamic rearrangements of the cytoskeleton in podocytes (12).

In CD2AP-deficient mice, CIN85 expression increases in podocytes and causes a profound defect in PI3K/AKT and Ras-ERK-MAPK signaling response after the stimulation of RTKs with various growth factors, such as fibroblast growth factor 4, at 3 weeks of age, which correlates with a rapid-onset nephrotic syndrome (13). In the presence of CIN85, which is involved in downregulation of RTKs, nephrin is internalized by endocytosis and ubiquitinated after stimulation with fibroblast growth factor 4. CIN85 binds directly to nephrin and podocin through its coiled-coil domain. Coexpression of CIN85 with CD2AP leads to a decreased binding of CIN85 to nephrin and podocin, which indicates a functional competition between CD2AP and CIN85, suggesting a novel role for CIN85 in slit diaphragm turnover and proteinuria (14). CIN85 is expressed in the membrane and nuclear fractions of CD2AP-deficient podocytes and is not detectable in wild-type podocytes. The 130-kDa form of CIN85 is mostly expressed in the cytosol and nucleus of wild-type podocytes and is barely detectable in CD2AP-deficient podocytes. CIN85 is SUMOylated by SUMO-1, -2, and -3, and the SUMOylation is enhanced in the presence of CD2AP. Conversion of lysine 598 to arginine in CIN85 completely abolishes SUMOylation and leads to increased binding of CIN85 to nephrin, indicating a novel role for CD2AP in regulating posttranslational modification of CIN85 (15).

In this issue of *Diabetes,* Teng et al. (16) demonstrated the novel role of CIN85 in the development of proteinuria of DN. CIN85 is upregulated in podocytes of glomeruli in experimental and human DN. In vivo studies using experimental DN showed that upregulated expression of CIN85 in podocytes of diabetic glomeruli coincides with downregulation of CD2AP. CIN85 is released from SUMO-1 and its interaction with nephrin is induced (Fig. 1). To investigate

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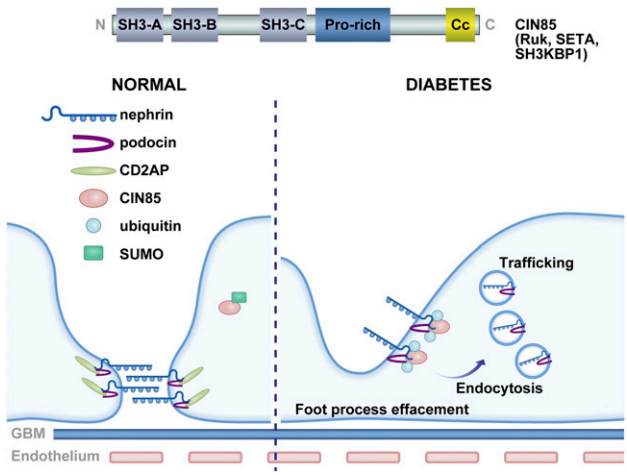

Figure 1—A schematic diagram of the domain structure of CIN85 and a proposed model of slit diaphragm turnover and trafficking of nephrin and podocin in diabetes. CIN85 contains three N-terminal SH3 domains, a centrally located proline-rich motif, and a COOH-terminal coiled-coil domain. In a normal condition, the slit diaphragm complexes, including CD2AP, nephrin, and podocin, are stabilized. The expression of CIN85 is low and SUMOylated. In a diabetic condition, the expression of CD2AP is decreased. CIN85 is upregulated and released from SUMO. CIN85 can bind to both nephrin and podocin and induce trafficking of both molecules. Cc, coiled-coil domain; GBM, glomerular basement membrane; Pro-rich, proline-rich motif.

the pivotal role of CIN85 in the development of proteinuria in DN, the authors used CIN85$^{\Delta ex2}$ mice, which lack the two major isoforms expressed in the kidney (CIN85-xl and CIN85) (17). Strikingly, in CIN85$^{\Delta ex2}$ diabetic mice, nephrin expression is preserved. Albuminuria and mesangial collagen IV expression are suppressed compared with diabetic wild-type mice. To prove that CIN85 is responsible for endocytosis of nephrin under high-glucose conditions, they investigated conditionally immortalized CIN85$^{\Delta ex2}$ podocytes and revealed impaired endocytosis compared with wild-type and CD2AP-deficient podocytes. Finally, impressive experiments using murine CIN85-mRNA–injected zebrafish were performed. CIN85-overexpressing zebrafish show foot process effacement and significant proteinuria compared with wild-type and CD2AP-overexpressing fish, which indicates a direct effect of CIN85 on podocyte damage–induced proteinuria.

The strength of the study by Teng et al. (16) is the demonstration of a directly responsible molecule for podocyte damage that develops both morphological and clinical manifestations of DN. Several molecules and signal pathways in podocytes, such as β-arrestin2 (18) and mTORC1 pathway (19), were shown to be involved in the development of DN. However, the detailed mechanism of these molecules to develop DN has yet to be fully examined. Conversely, the study by Teng et al. (16) proposed a direct cause-effect relationship between podocyte change and proteinuria through CIN85 function.

Teng et al. (16) claim that CIN85 expression shows the potential to be a novel target for antiproteinuric therapy in DN. Therefore, we believe that several questions below should be clarified in the future. In reality, in the UK Prospective Diabetes Study (UKPDS), 24.9% of patients developed microalbuminuria within 10 years of diagnosis of type 2 diabetes (20). This means that there are more than a few patients whose kidneys are resistant to hyperglycemia-induced damage. Is the current high glucose–CIN85 story consistent with the long-term and variety of DN natural history? Is CIN85 expressed in the early stages of human DN? Are there any differences of CIN85's role in developing DN between type 1 and 2 diabetes? Are there any factors, such as hypertension or drugs other than high glucose and growth hormone, that affect CIN85 expression? It is also tempting to investigate the relationship between CIN85/CD2AP complex gene variants and their function and susceptibility to the development of DN.

In conclusion, CIN85/CD2AP can be a responsible complex for the deterioration of podocyte integrity and proteinuria in diabetes. The study by Teng et al. (16) provides new insights regarding podocytopathies as causal in the development of DN. The regulation of endocytosis in podocytes should be further clarified to find out more candidates for pharmacological intervention.

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