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Role of Megalin and Cubilin in the Metabolism of Vitamin D_3

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Abstract: Vitamin D deficiency is associated with various medical conditions including musculoskeletal disorders, infection, metabolic diseases, and cardiovascular disease. Megalin and cubilin, endocytic receptors in proximal tubule cells, are involved in the reabsorption of vitamin D binding protein from glomerular filtrates and the subsequent intracellular conversion of 25-hydroxyvitamin D_3 to biologically active 1α,25-dihydroxyvitamin D_3. Dysfunction of these receptors, which is commonly found in patients with diabetic nephropathy, even at early stages, may explain why vitamin D deficiency is often complicated in these patients. Therapeutic strategies to protect the functions of these receptors from injury could be used to prevent vitamin D deficiency and its related disorders.
Key Words: Chronic kidney disease, Cubilin, Diabetic nephropathy, Megalin, Vitamin D deficiency.

Vitamin D is obtained exogenously through dietary intake and is synthesized endogenously in the skin, where 7-dehydrocholesterol (pro-vitamin D_3) is converted to pre-vitamin D_3 by UV radiation. Pre-vitamin D_3 then undergoes non-enzymatic isomerization to form cholecalciferol, or vitamin D_3. This enters the circulation and is carried by vitamin D binding protein (DBP) to the liver where it is hydroxylated by 25-hydroxylase to 25-hydroxyvitamin D_3 ($25(OH)D_3$). Finally, $25(OH)D_3$ is again transported by DBP to the kidney, where it is hydroxylated by 1α-hydroxylase to 1α,25-dihydroxyvitamin D_3 ($1,25(OH)_2D_3$), which is the hormonally active form of vitamin D (1). It has recently been suggested by *in vitro* studies that conversion of $25(OH)D_3$ to $1,25(OH)_2D_3$ may also occur in extra-renal cells, including keratinocytes, bone, placenta, prostate, macrophages, T-lymphocytes, and dendritic cells (2).

Vitamin D deficiency is associated with various medical problems, including musculoskeletal disorders, infection, metabolic diseases, and cardiovascular disease (3). It develops early in the course of chronic kidney disease (CKD), especially in diabetic nephropathy. Treatment with the activated vitamin D analogue calcitriol was shown to improve the survival of patients suffering from this disorder (4), while a recent meta-analysis of randomized controlled studies of vitamin D supplementation in the general population suggests that it also decreases total mortality rates (5).

In this review, we focus on the renal mechanism of vitamin D_3 metabolism mediated by megalin and cubilin, as well as its disorder in CKD, particularly diabetic nephropathy.

MEGALIN AND CUBILIN: ENDOCYTIC RECEPTORS IN PROXIMAL TUBULE CELL (PTC) APICAL MEMBRANES

Megalin is a large (~600 kDa) glycoprotein member of the low-density lipoprotein receptor family that is primarily expressed in clathrin-coated pits (6,7). Megalin plays a critical role in the reabsorption and metabolism of glomerular-filtered substances, including albumin and low molecular weight proteins. Megalin–ligand complexes are internalized via clathrin-coated pits mediated by multiple

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intracellular adaptor proteins such as disabled 2 (Dab2) and motor molecules to form endosomal vesicles (8). Acidification of the intravesicular lumen dissociates the ligands from megalin and they are transported to lysosomes for degradation or storage, or translocated to the cytosol for further processing. Megalin cooperates with cubilin, another endocytic receptor of PTCs.

Cubilin is a 460-kDa peripheral glycoprotein lacking transmembrane and intracellular segments, but is anchored to apical PTC membranes (7). It was originally identified as the receptor for the intrinsic factor–vitamin B_{12} complex (9). Cubilin gene defects are the cause of hereditary megaloblastic anemia 1 and Imerslund–Gräsbeck syndrome, also known as selective vitamin B_{12} malabsorption with proteinuria (10). Cubilin is also involved in the absorption of various protein ligands present in glomerular filtrates, including albumin and transferrin (7). It is bound by amnionless, a 50-kDa membrane protein, forming the complex CUBAM (11).

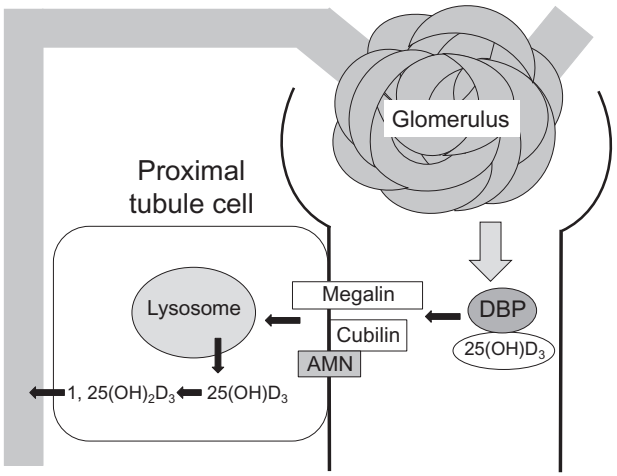

FIG. 1. Involvement of megalin and cubilin in vitamin D_3 metabolism in proximal tubule cells: megalin and cubilin take up vitamin D-binding protein (DBP) from glomerular filtrates. Following degradation of DBP in lysosomes, 25-hydroxyvitamin D_3 ($25(OH)D_3$) is converted to 1α,25-dihydroxyvitamin D_3 ($1,25(OH)_2D_3$) intracellularly and released into the circulation. AMN, amnionless.

INVOLVEMENT OF MEGALIN AND CUBILIN IN VITAMIN D_3 METABOLISM

A breakthrough in unveiling the link between vitamin D_3 metabolism and renal receptor-mediated endocytosis was the finding that megalin mediates the uptake of DBP from glomerular filtrates and that the process is essentially involved in the conversion of $25(OH)D_3$ to $1,25(OH)_2D_3$, a biologically active form (Fig. 1) (12,13). The relevance of DBP in the metabolism and activation of vitamin D was also supported by results obtained using DBP knockout mice (14). These findings challenged the previous free hormone hypothesis, which stated that the biological activity of a hormone is mediated by its unbound (free) form rather than its protein-bound forms in the plasma (15). Subsequently, cubilin was shown to be another endocytic receptor for DBP, with its genetic defects causing urinary loss of DBP and a decrease in plasma $25(OH)D_3$ and $1,25(OH)_2D_3$ levels in humans and dogs (16). A recent study, however, found that urinary DBP levels are not increased in cubilin knockout mice, suggesting that the mouse mechanism of DBP processing might differ (17).

IMPAIRED ENDOCYTIC FUNCTIONS OF PTCS IN DIABETIC NEPHROPATHY

Decreased megalin expression in PTCs has been observed in the early diabetic stages of experimental animals (18). Megalin function is also believed to be impaired in the early stages of diabetic nephropathy, since low molecular weight proteinuria is frequently observed in such patients (19).

Cellular expression of megalin was found to be downregulated by TGF-β (18). We also found that megalin expression in cultured PTCs is upregulated following treatment with insulin or high-concentration glucose. Conversely, it is downregulated by angiotensin II (20). Furthermore, we demonstrated competitive cross-talk between angiotensin II type 1 receptor- and insulin-mediated signaling pathways in the regulation of megalin expression in the cells (20). Angiotensin II may be a major factor in suppressing megalin expression in the early stages of diabetic nephropathy since the intrarenal renin–angiotensin system is activated in the disease (21).

The functions of cubilin may also be impaired in early diabetic nephropathy as urinary excretion of transferrin, an endocytic ligand of cubilin, is significantly increased in patients with this disease (22).

VITAMIN D DEFICIENCY AND ITS LINK WITH PTC ENDOCYTIC DYSFUNCTION IN DIABETES

The type 2 diabetes animal model, Zucker fatty rats, shows reduced $25(OH)D_3$ and $1,25(OH)_2D_3$ serum levels, which are associated with increased urinary excretion of these vitamin D metabolites and DBP and reduced renal expression of megalin and

Dab2 (23). It was also found that increased urinary excretion of DBP is associated with vitamin D deficiency in patients with type 1 diabetes (24). Interventional studies should therefore be conducted to investigate whether vitamin D deficiency is prevented by maintaining the function of megalin and cubilin in patients with CKD, especially those with diabetic nephropathy.

EFFECTS OF VITAMIN D RECEPTOR ACTIVATION ON THE REDUCTION OF ALBUMINURIA IN DIABETIC NEPHROPATHY

Recently, a randomized controlled trial showed that the daily administration of 2 μg paricalcitol, an analogue of the active form of vitamin D_3, to renin-angiotensin-aldosterone inhibition lowered residual albuminuria in patients with diabetic nephropathy. A lower dose (1 μg/day) of the drug produced no favorable effects (VITAL study) (25). The mechanisms of paricalcitol action on the kidney remain unknown, so it would be of interest to investigate whether it is taken up via the megalin–cubilin system and if it acts on the PTC vitamin D receptor. High-dose paricalcitol may have been needed to generate such a beneficial effect in patients with diabetic nephropathy because of the reduced renal functions of megalin and cubilin in these patients.

CONCLUSIONS

The endocytic PTC receptors megalin and cubilin are involved in the metabolism of vitamin D by reabsorbing DBP from glomerular filtrates. Dysfunction of these receptors is likely to be associated with the development of vitamin D deficiency in patients with CKD, in particular those with diabetic nephropathy. Therapeutic strategies to protect the functions of these receptors from injury could be investigated to prevent vitamin D deficiency and its related disorders.

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