

Review Article

Molecular Mechanisms of Receptor-Mediated Endocytosis in the Renal Proximal Tubular Epithelium

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Receptor-mediated endocytosis is a pivotal function of renal proximal tubule epithelial cells (PTECs) to reabsorb and metabolize substantial amounts of proteins and other substances in glomerular filtrates. The function accounts for the conservation of nutrients, including carrier-bound vitamins and trace elements, filtered by glomeruli. Impairment of the process results in a loss of such substances and development of proteinuria, an important clinical sign of kidney disease and a risk marker for cardiovascular disease. Megalin is a multiligand endocytic receptor expressed at clathrin-coated pits of PTEC, playing a central role in the process. Megalin cooperates with various membrane molecules and interacts with many intracellular adaptor proteins for endocytic trafficking. Megalin is also involved in signaling pathways in the cells. Megalin-mediated endocytic overload leads to damage of PTEC. Further studies are needed to elucidate the mechanism of megalin-mediated endocytosis and develop strategies for preventing the damage of PTEC.

1. Introduction

Renal proximal tubular epithelial cells (PTECs) are involved in a variety of vital functions. Of these, receptor-mediated endocytosis is a pivotal function of the cells to reabsorb and metabolize proteins and other substances in glomerular filtrates. Megalin is a membrane receptor that plays a central role in the endocytic functions of PTEC. Megalin cooperates with various molecules in the cells, taking up ligands into the endocytic pathway to lysosomes, as well as mediating signal transduction. In this review, we focus on recent progress in the research on megalin and its associated molecules. We also discuss how impaired or overloaded endocytosis induces PTEC damage which is tightly associated with the onset of proteinuria and the development of chronic kidney disease (CKD).

2. Megalin: A Major Endocytic Receptor in PTEC

Megalin is a large (~600 kDa) glycoprotein member of the low-density lipoprotein (LDL) receptor family [1, 2] that

is primarily expressed at clathrin-coated pits and partly at microvilli of PTEC (Figure 1) [3, 4]. Megalin contains a huge extracellular domain responsible for its multispecific properties. The domain consists of 4398 amino acids (in humans) and is made by three types of repeats which are characteristic of the LDL receptor family: (1) 36 cysteine-rich complement-type repeats organized in four clusters, (2) 16 growth factor repeats separated by 8 YWTD containing spacer regions involved in pH dependent release of ligands in endosomal compartments [5], and (3) a single epidermal growth factor-like repeat. The extracellular domain is followed by a single transmembrane segment and a cytoplasmic domain of 209 amino acids. The cytoplasmic tail contains two endocytic motifs (NPXY) mediating clustering into clathrin coated pits and an NPXY-like motif (NQNY) involved in apical sorting of the receptor [6] as well as other protein interaction motifs (SH3 and PDZ domains) and phosphorylation sites [1, 2]. The physiological potential of these regulatory motifs has not yet been fully understood.

Megalin plays a critical role in the reabsorption of glomerular-filtered substances including albumin and

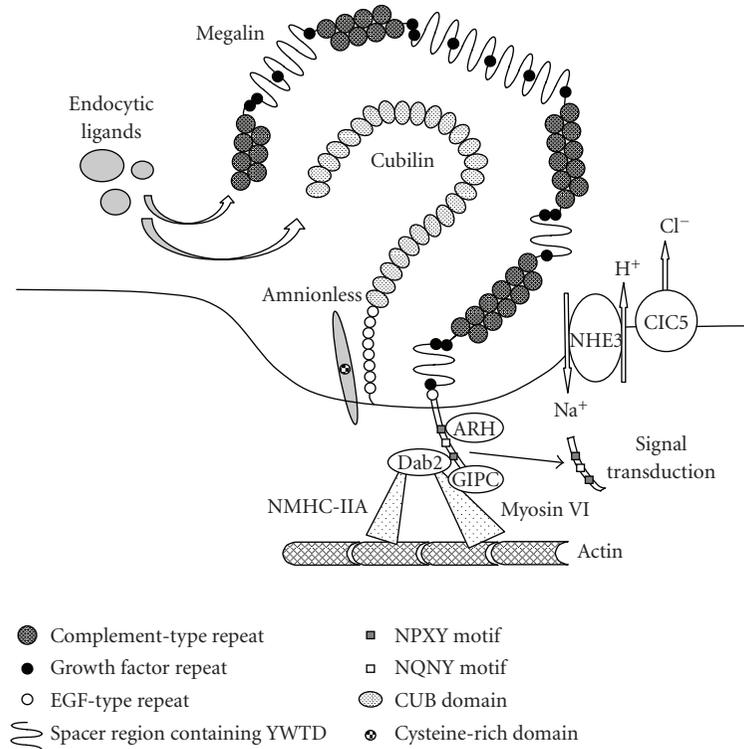


FIGURE 1: Megalin and its associated molecules involved in receptor-mediated endocytosis in PTEC. On the apical membrane of PTEC, various molecules are involved in the process of receptor-mediated endocytosis. Megalin, playing a central role in the process, cooperates with other membrane proteins such as the cubilin-amnionless complex (CUBAM), NHE3, and CIC5. Megalin and CUBAM directly bind a variety of ligands, whereas NHE3 and CIC5 are involved in endosomal acidification, which is important for further processing of endocytosed proteins. Megalin also interacts with intracellular adaptor proteins such as ARH, Dab2, and GIPC. Dab2 binds to motor proteins, myosin VI, and NMHC IIA, which may mediate endocytic trafficking of the molecular complexes through actin filaments. The cytoplasmic tail of megalin is released from the membrane by γ -secretase and is involved in intracellular signal transduction.

low-molecular-weight proteins. Also, megalin may take up proteins that are released by PTEC to the apical tubular space. Megalin knockout mice display low-molecular-weight proteinuria and albuminuria [7]. Furthermore, patients with Donnai-Barrow and facio-oculo-acoustico-renal syndromes, caused by mutations in the megalin gene, show increased urinary excretion of albumin and low-molecular-weight proteins [8]. In this process, megalin mediates the conservation of carrier bound vitamins and trace elements filtered by glomeruli, including vitamin D [9], vitamin A [10], vitamin B₁₂ [11], and iron [12]. Megalin cooperates with a variety of molecules at the apical membranes and also in the cytoplasm of PTEC (Figure 1) as described in the next section.

3. Molecules Associated with Megalin's Functions in PTEC

3.1. Cubilin-Amnionless Complex (CUBAM). Cubilin is a 460-kDa peripheral glycoprotein, thus lacking transmembrane and intracellular segments, but anchored to the apical membranes in PTEC. It was originally identified as the receptor for intrinsic factor-vitamin B₁₂ [13, 14], and its gene defects are the causes of hereditary megaloblastic

anaemia 1 or Imerslund-Gräsbeck syndrome (selective vitamin B₁₂ malabsorption with proteinuria) [15]. Cubilin is also involved in the absorption of various protein ligands present in glomerular filtrates, including albumin, transferrin, and vitamin D-binding protein [4]. Cubilin is known to interact with megalin for its endocytic functions [12, 16]; however, it is bound more firmly by a protein called amnionless, forming a complex named CUBAM, to be translocated to the plasma membrane [17, 18]. Amnionless, a 38–50 kDa membrane protein with a single transmembrane domain, was initially identified as a component for the normal development of the trunk mesoderm derived from the middle streak [19]. Its gene defects also cause hereditary megaloblastic anaemia [20]. However, the role of amnionless in PTEC is not fully identified.

3.2. Na⁺/H⁺ Exchanger Isoform 3 (NHE3). NHE3, the main NHE isoform in PTEC, mediates isotonic reabsorption of approximately two thirds of the filtered NaCl and water, the reabsorption of bicarbonate, and the secretion of ammonium [21]. It also contributes to the reabsorption of filtered citrate, amino acids, and oligopeptides by providing H⁺ used for the H⁺-coupled cotransporters. Enhanced NHE3 activity is assumed to be a factor for increased Na⁺ reabsorption

and the development of hypertension in diabetes. NHE3 was reported to interact with megalin in intermicrovillar clefts of PTEC [22, 23]. After endocytosis with megalin, NHE3 is postulated to utilize the outward transvesicular Na^+ gradient of endocytic vesicles and early endosomes to drive inward movement of H^+ and endosomal acidification, which is important for dissociating reabsorbed ligand proteins from megalin for further processing.

3.3. *ClC-5*. *ClC-5* is a 746-amino acid protein originally assumed to belong to the voltage-gated chloride channel family [24], but more recent evidence suggests that it may function as an H^+/Cl^- exchanger [25]. In kidney, *ClC-5* is highly expressed in PTEC and α and β intercalated cells of collecting ducts [26]. In PTEC, *ClC-5* is located at the apical endosomes together with electrogenic V-type H^+ -ATPases [26], where it has a complementary function in endosomal acidification [27]. The physiological relevance of *ClC-5* in renal functions came into view when mutations in the *CLCN5* gene were identified in patients with Dent's disease, an X-linked renal tubular disorder [26]. This disorder is characterized by low molecular weight proteinuria, hypercalciuria, nephrocalcinosis, nephrolithiasis, aminoaciduria, phosphaturia, glycosuria, and renal failure [28]. The precise mechanism of this abnormality is not entirely clear but possibly results from defective acidification and/or reduced expression of megalin and cubilin in PTEC [29, 30].

3.4. *Intracellular Adaptor Proteins*. Various sorting and signaling proteins bind to megalin's cytoplasmic tail such as JIP1 and JIP2, SEMCAP-1 (GIPC), ANKRA, Dab2, PDS-95, MegBP, and ARH [31–37]. ARH and Dab2 are components of the clathrin coat, and they bind to the first and third NPXY motif of megalin, respectively, through their PTB domains [33, 37]. ARH and Dab2 are known to interact with motor proteins as described below. Dab2 is also known to mediate signal transduction [38, 39].

4. Regulation of Megalin Expression

Cellular expression of megalin was found to be downregulated by the action of TGF β [40]. We also found that megalin expression is upregulated in cultured PTEC by treatment with insulin or high-concentration glucose (17.5 mM), whereas it is downregulated by angiotensin II [41]. Furthermore, we demonstrated that there is competitive cross talk between angiotensin II type 1 receptor and insulin-mediated signaling pathways in the regulation of megalin expression in the cells, suggesting a counter-balanced mechanism that regulates megalin expression and functions in PTEC [41].

Decreased megalin expression in PTEC has been found in the early diabetic stages in experimental animals [40, 42]. It is also suggested that the functions of megalin are impaired in patients in the early stages of diabetic nephropathy, since low-molecular-weight proteinuria are frequently observed in patients at these stages [43, 44]. Thus,

the altered regulation of megalin expression and functions must be significantly responsible for the early development of proteinuria/albuminuria in diabetic patients. The mechanisms of the regulation remain to be further investigated.

5. Regulation of Megalin Transport by Motor Proteins

The mechanisms of intracellular transport of megalin are largely unknown. Reverse-direction molecular motor myosin VI was found to be linked to Dab2 and GIPC, which binds to the cytoplasmic tail of megalin, and is assumed to be involved in the endocytosis in PTEC [45]. However, myosin VI knockout mice, used as an animal model for deafness, showed no apparent renal manifestation presenting proteinuria [46].

We recently identified that another motor protein, nonmuscle myosin heavy chain IIA (NMHC IIA), binds to Dab2 and is involved in megalin-mediated endocytosis [47]. Genetic alterations of NMHC-IIA are known to cause inherited human diseases, known as MYH9 disorders, which are characterized by giant platelets, thrombocytopenia, and granulocyte inclusions [48, 49]. The spectrum of diseases due to mutations in the gene includes May-Hegglin anomaly, Sebastian syndrome, Fechtner syndrome, and Epstein syndrome [48–51]. It has been also reported that all of these disorders are related to development of kidney disease [50, 52]. The manifestation of kidney disease in MYH9 disorders indicates the importance of NMHC-IIA in maintaining normal kidney functions, which has been also verified by two recent genomewide scan analyses [53, 54].

Another megalin-binding adaptor protein ARH also associates with motor and centrosomal proteins and is involved in centrosome assembly and cytokinesis [55]. The relevance of the adaptor protein's association with such molecules in the regulation of megalin transport remains undetermined.

6. Overloaded Endocytosis-Induced PTEC Injury in CKD

Overloaded endocytosis in PTEC due to increased glomerular protein filtration has been postulated to be a cause of tubulointerstitial injury. Megalin is identified as the key molecule to initiate the pathogenic process [56]. In diabetes, advanced glycation endproducts (AGEs) are generated in the circulation and involved in a variety of cellular damage [57]. Megalin also mediates the endocytosis of glomerular-filtered AGE in PTEC [58, 59], which causes toxicity in the cells [60, 61]. In metabolic syndrome or dyslipidemia, free fatty acids are delivered to PTEC with the carrier proteins such as albumin or liver-type fatty acid binding protein [62]. Metabolically overloaded PTECs are activated to express proinflammatory cytokines, such as MCP1 and TNF α , and lead to apoptosis [56] or epithelial-mesenchymal transition [63, 64].

7. Handling of Albumin in PTEC, Related to the Mechanism of Albuminuria

Albumin (~69 kDa) is the most abundant circulating protein, carrying a variety of substances in plasma. Glomerular albumin filtration is assumed to be 3–6 g/d in humans [65]. Only negligible amounts of albumin are detected in urine, and the substantial remaining of glomerular-filtered albumin is reabsorbed in PTEC via endocytosis, mediated by megalin and CUBAM. Albuminuria is an important clinical sign of kidney disease such as diabetic nephropathy [66, 67] as well as a risk marker of cardiovascular disease (CVD) [68, 69]. Impaired endocytic functions of PTEC for albumin are relevant to the mechanisms of albuminuria.

After endocytosis, albumin is considered to be transferred to lysosomes for degradation to amino acids [70]. On the contrary, the presence of a retrieval or transcytic pathway of albumin in PTEC is suggested [71]. A recent analysis using neonatal Fc receptor knockout mice supports the retrieval pathway in PTEC where the receptor appears to play a critical role to reclaim albumin from the glomerular filtrates [72].

The association of albuminuria with the development of CVD may be related to the impairment of metabolic or synthetic functions of PTEC that may contribute to systemic vascular damage. For instance, vitamin D deficiency, which is caused by megalin dysfunction, is independently associated with increased cardiovascular mortality [73, 74]. Selenoprotein P, a major carrier of selenium, is taken up by megalin [75] and provides selenium for synthesizing glutathione peroxidase 3 (GPx3) in PTEC [76, 77]. GPx3 is secreted into the extracellular space from where it enters the blood and acts as antioxidant [78]. Therefore, reduced uptake of selenoprotein P in PTEC due to impaired megalin function may result in decreased GPx3 synthesis by the cells and may be associated with the development of vascular diseases.

8. Megalin-Mediated Signaling

Biemesderfer and his colleagues identified that megalin undergoes regulated intramembrane proteolysis as some other membrane proteins such as those belonging to the Notch and amyloid precursor protein families [79, 80]. They showed (1) that high levels of γ -secretase are expressed in the brush border and endocytic pathway of PTEC where it colocalizes with megalin, (2) that megalin is subjected to PKC-regulated, metalloprotease-mediated ectodomain shedding that produces a 35 to 40 kDa megalin COOH-terminal fragment (MCTF), and (3) that the MCTF is membrane bound and is constitutively processed by γ -secretase activity [81]. They also found evidence suggesting that the COOH-terminal domain of megalin regulates megalin and NHE3 gene expression [82]. These findings strongly indicate that megalin is not only involved in scavenging functions in PTEC but also participate in the signal transduction in the cells.

9. Conclusions

Megalin, an endocytic receptor, mediates the conservation of nutrients and carrier bound vitamins and trace elements in

glomerular filtrates via interaction with various molecules in PTEC. Megalin also plays a critical role in the uptake of pathological substances or overloaded endocytosis that may lead to the cellular damage. Megalin-mediated signaling transduction may be also involved in the process. Further studies are needed to elucidate the molecular mechanism fully and develop strategies for preventing PTEC damage.

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