Protein trafficking defects in inherited kidney diseases

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Introduction

Proteins are responsible for nearly every task of cellular life. 'Protein trafficking' refers to the journey of protein from a simple polypeptide chain translated from the mRNA through its post translational modifications in the endoplasmic reticulum (ER) and golgi apparatus before being finally dispatched for their respective functions. This entire process is depicted in figure 1. The process starts with protein folding in the ER which is a complex process tightly regulated by the cell machinery as explained below. From the ER, properly folded proteins reach the golgi apparatus where they undergo glycosylation, get sorted for transport to their intended destination within membrane bound vesicles. (1)

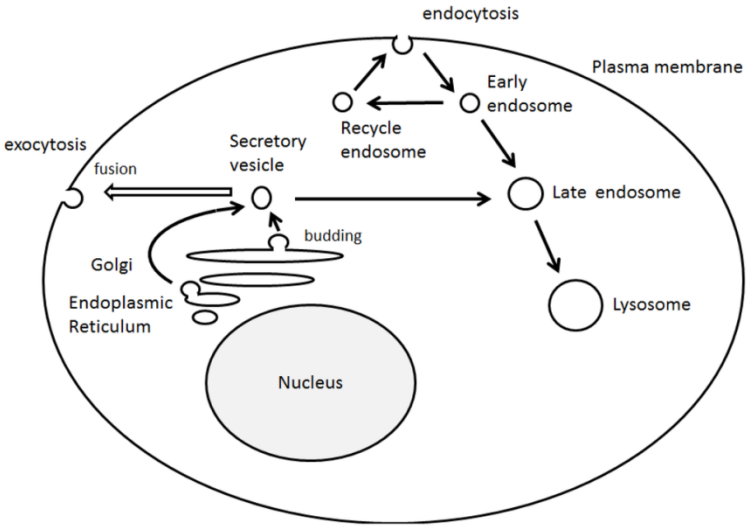


Figure 1: Pathway of intracellular protein trafficking tracing the orderly movement of proteins from synthesis to modifications in the endoplasmic reticulum and golgi apparatus and final packing into vesicles for transport to either lysosomes or exocytosis into extracellular space.

Protein folding within the endoplasmic reticulum is the "Achilles heel" of the process of protein trafficking.(2) We shall see the process of protein folding and misfolding in detail as it forms the backbone for understanding inherited diseases associated with protein trafficking.

Protein folding and misfolding

The balance between protein synthesis, folding and degradation is tightly regulated and is constantly challenged by genetic and environmental stress factors. The endoplasmic reticulum (ER) is the major sub-cellular organelle involved in protein folding and maturation and about one-third of the total proteome is synthesized in the ER.

Each protein exists as a random coil (unfolded polypeptide) when translated from a mRNA sequence and in the ER they undergo glycosylation and disulfide bond formation coordinated by folding enzymes and molecular chaperones to attain the final three dimensional stable folded state following which they are transported to the golgi apparatus. The cell is equipped with a number of 'quality control' mechanisms to minimize misfolding and dispose of misfolded proteins prior to aggregation. The two major defenses against misfolding are chaperones and proteasomes. Unfolded and partially folded protein chains expose hydrophobic amino acid residues that can give rise to aggregation. Molecular chaperones shield these regions preventing aggregation. If proteins are not folded properly they undergo "retrotranslocation" to get degraded by the proteasome. This process is termed ER associated degradation (ERAD) as depicted in figure 2.(3)

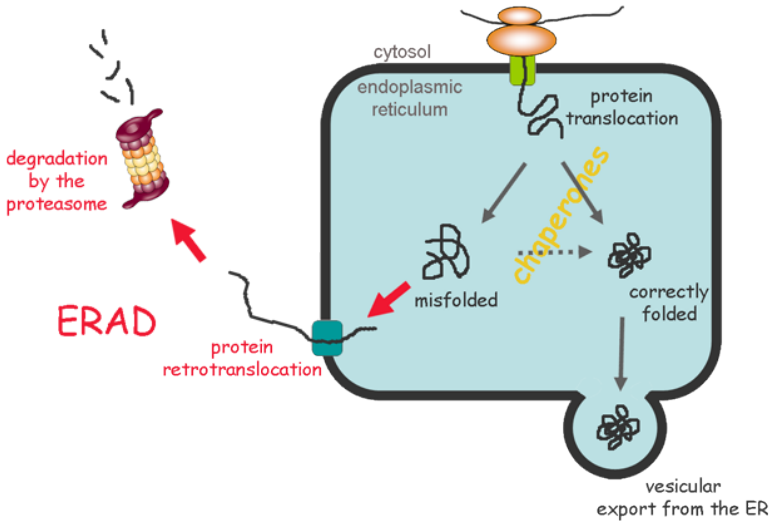


Figure 2: Graphic illustration of protein folding in endoplasmic reticulum and outcome of either export of correctly folded protein or degradation of misfolded proteins by proteasome. ERAD, endoplasmic reticulum associated degradation

Accumulation of misfolded proteins within the ER leads to the cellular condition termed ER stress which in turn initiates a series of adaptive mechanisms that together are termed as the unfolded protein response (UPR).(4) UPR tries to restore cellular function by halting protein translation, increasing the synthesis of molecular chaperones which prevent further misfolding and promote degradation of misfolded proteins by the ERAD pathway. If by this initial response, UPR isn't able to achieve its objectives, the process of apoptosis is initiated through Ire and PERK receptors.

Proteostasis in kidney disease

Protein homeostasis or proteostasis is a sophisticated network composed of intricate linkages between cytosolic components, endoplasmic reticulum and mitochondrial pathways involved in regulating protein structure and function.(5) Crosstalk between the various components ensures normal functioning of the cell. Hypoxia, glycative stress and oxidative stress induce stress signals that derange ER proteostasis in glomerular and tubulointerstitial cells and lead to alterations in the structure and function of kidney cells. This has been shown in rat models of glomerulonephritis wherein preconditioning with ER stress ameliorated glomerular damage by damping the

excessive UPR activation caused by disease induction.(6) This section will focus on the effects of ER stress on various parts of the glomeruli.

Podocytes

Mutations of podocyte slit diaphragm components, namely nephrin, α-actinin-4 and CD2-associated protein (CD2AP) are seen in congenital nephritic syndromes.(7) These congenital syndromes are associated with the accumulation of misfolded proteins in the ER causing disruption of the slit diaphragm leading to proteinuria. Mutation of laminin subunit β2 seen in glomerular basement membrane (GBM) is characterized by podocyte ER stress.(8) C5-9 membrane attack complex & calcium entry via transient receptor protein 6 (TRPC6) also induce UPR activation.(9) Activation of rapamycin-sensitive protein kinase TORC1 and increased expression of monocyte chemoattractant protein 1 (MCP-1) induce ER stress in diabetic nephropathy which causes podocyte injury and consequently, proteinuria.

Tubular cells

Tubular cells of the kidney have an active UPR pathway. Imbalance in the UPR pathway due to proteinuria, hyperglycaemia, uremic toxins, nephrotoxins and oxidative stress initiate apoptosis and accelerate the progression of kidney disease. ER proteostasis is closely linked to tubular ageing as GRP78 expression seen with adaptive UPR response is suppressed while CHOP expression seen with pro-apoptotic UPR is enhanced with exposure of tubular cells to proteinuria in the elderly.(10)

Interstitial cells

The prototype interstitial cell namely pericytes are involved in tubulointerstitial fibrosis. ER stress with altered proteostasis forms the basis for fibrosis via activation of ER-resident protein ERP57.(11) Rat models have shown UPR activation to be associated with fibrosis and this has been attenuated by candesartan.(12) Altered proteostasis forms the basis of anemia of chronic kidney disease as it is mediated by ATF4 which binds to enhancer region of EPO gene thereby reducing EPO synthesis.(13)

Protein trafficking & inherited renal disease

For the approximately 5,400 known Mendelian disorders in humans the causative genes have been identified in only about 2,600 diseases with around 120 genes being associated with renal diseases. Of these monogenic diseases around one-third of them interfere directly with protein trafficking.(14) Abnormal localization and/or aggregation of protein has become recognized as a critical issue in a growing number of inherited diseases. Some of the common inherited diseases associated with protein trafficking with respect to the nephron segment affected are depicted in figure 3.

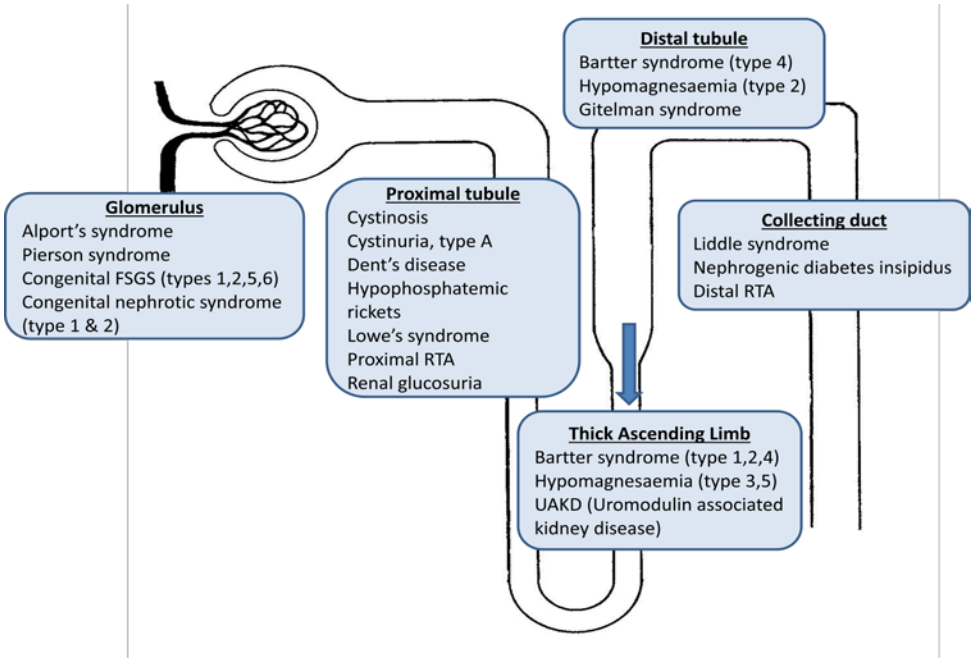


Figure 3: Mendelian renal diseases associated with defective protein trafficking

Diseases like Fabry disease, cystic fibrosis, atypical Hemolytic Uremic Syndrome (aHUS) and primary hyperoxaluria affect proteostasis in multiple organs and result in secondary damage to the nephron. Retention of proteins in the endoplasmic reticulum is the predominant mechanism for disease process. The other disorders of protein trafficking include mistargeting of proteins, defective endocytosis and defective degradation.(15)

ER retention

Mutations which affect folding of proteins result in accumulation of misfolded proteins in the endoplasmic reticulum resulting in ER stress thereby activating the UPR as explained earlier. Classical examples of diseases due to ER retention include nephrogenic diabetes insipidus and congenital nephrotic syndromes.

Nephrogenic diabetes insipidus (NDI) is a disease of water balance characterized by defective urinary concentrating mechanism. It can be either congenital or acquired. Loss of function mutations of the arginine vasopressin V2 receptor (AVPR2) gene is responsible for nearly 90% of the cases of congenital NDI and has X-linked inheritance. Most of the remaining forms of hereditary NDI are caused by mutations of the aquaporin 2 (AQP2) gene located on chromosome 12, and both recessive and dominant inheritance patterns have been reported.(16) Water reabsorption in the principal cells of the collecting duct occurs due to the effect of arginine vasopressin (AVP) on AVPR2 which activates adenyl cyclase and protein kinase A stimulating insertion of AQP2 channels into the apical membrane as illustrated in figure 4.

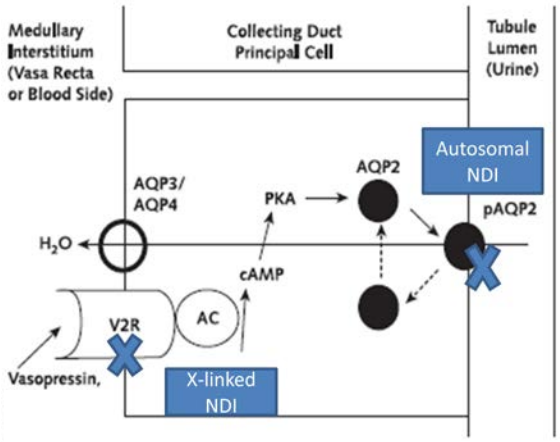


Figure 4: Role of AVP in water absorption in collecting duct and sites of mutation (blue cross) in congenital NDI. AC, adenyl cyclase; PKA, protein kinase A; AQP, aquaporin; V2R, vasopressin V2 receptor

Depending on the molecular mechanism the mutations causing NDI are divided into various classes as summarized in table 1.(17) Patients with similar mutations differ clinically with regard to the severity of symptoms and response to therapy. This is because some patients have partial trafficking of protein with some residual function and so respond to high dose vasopressin analogues in contrast to those with full blown NDI.

	Type of mutations	Effect of mutation
X-Linked NDI		
Class I	Frameshift/Nonsense	Affects mRNA synthesis/processing causing truncated protein
Class II – most common (> 50%)	Missense/in-frame deletion or insertion	Misfolded proteins with ER retention
Class III	Missense/in-frame deletion	Interfere with G protein or AVP binding
Class IV	Missense	Protein missorting
Autosomal NDI		
Autosomal Dominant NDI	Missense	Mislocalisation of AQP2
Autosomal Recessive NDI	Missense	Protein misfolding with ER retention

Table 1: Types of Nephrogenic Diabetes Insipidus (NDI) and causative mutations

Uromodulin associated kidney disease (UKAD) is autosomal-dominant slowly progressive disorder caused by mutations of the uromodulin (*UMOD*) gene characterized by hypouricosuric hyperuricemia, gout, defects in urine concentrating ability and progressive renal dysfunction. UMOD mutations result in protein misfolding, ER retention and gain-of-function effect leading to progressive dysfunction of the thick ascending limb of Henle with consequent interstitial fibrosis.(18)

The role of unfolded protein response in causing ER retention in congenital nephrotic syndromes has been explained earlier. In Alport's syndrome, mutations in type IV collagen result in ER stress finally leading to podocyte apoptosis and foot process effacement.

Protein mistargeting

Proteins are genetically programmed to reach a predestined destination to carry out their normal functions. Dysregulation of these 'targeting' signals has adverse consequences as seen in primary hyperoxaluria and distal renal tubular acidosis.

Primary hyperoxaluria type 1 (PH1) is caused by a deficiency of the liver peroxisomal enzyme alanine:glyoxylate-aminotransferase (AGT), which catalyzes the conversion of glyoxylate to glycine. When this is absent, glyoxylate is converted to oxalate, which forms insoluble calcium salts. Patients have an inexorable decline in renal function as a result of progressive nephrolithiasis/nephrocalcinosis, with eventual progression to oxalosis and ESRD. Most individuals with classic PH1 who have residual AGT enzymatic activity have a unique protein trafficking defect in which functional AGT enzyme is synthesized in adequate amounts but approximately 90% of the enzyme produced is mislocalized to mitochondria and only approximately 10% is properly localized in the peroxisomes. Individuals with such a protein-trafficking defect have classic PH1 despite the presence of residual AGT activity. Therapy is directed at preventing renal stones with high fluid intake and alkalinization of urine. Pyridoxine therapy increases intracellular pyridoxal phosphate which acts as a chaperone increasing expression and import of peroxisomes thereby partially reversing the defect. This discovery has paved the way for studies on other possible chaperones targeting peroxisomal transport.(19)

Distal renal tubular acidosis (dRTA) is caused by impaired distal acidification due to failure of the distal nephron to secrete H+. Intercalated cells in the collecting tubules secrete protons into the tubular lumen through H+-ATPases functionally coupled to the basolateral anion exchanger 1 (AE1). Mutations in the AE1, the gene that encodes the Cl^-/HCO_3^-exchanger, usually presents as dominant dRTA. Trafficking defects in mutant protein rather than lack of function is the major pathophysiological mechanism underlying dRTA. AE1 mutations are of two types. The first type as in S613F mutation causes ER retention and consequent degradation.(20) The second type of mutation as seen in R901X mutation causes protein mistargeting. Mistargeted AE1 mutants leads to apical mislocalization thereby causing bicarbonate secretion into the collecting duct lumen. At times they are trafficked to the basolateral membrane or the golgi complex. Some mutations cause carbonic anhydrase II (CA) deficiency which has features of both proximal & distal RTA. The normal process of distal acidification and mutations seen in dRTA is shown in figure 5.(21)

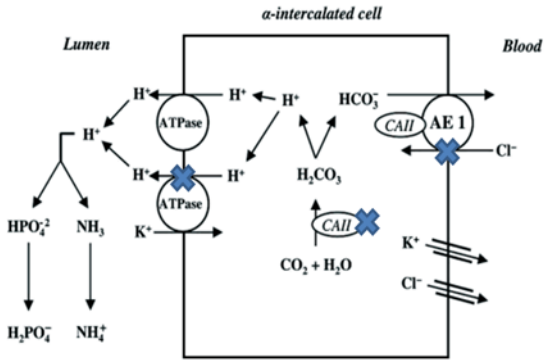


Figure 5: Normal process of distal acidification and sites of mutation in distal RTA (blue cross)

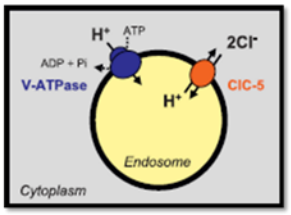
Identification of these pathogenetic mechanisms led to studies exploring the possibility of 'trafficking rescue' with DMSO, glycerol and VX-809. Interventions to prevent interaction of mutant protein with calnexin and Hsc70 are being studied as treatment options.

Defective endocytosis

Endocytosis is important for plasma membrane homeostasis, modulation of signal transduction and nutrient uptake. It involves internalization of various components into endosomes and lysosomes. Defective endocytosis forms the pathophysiological basis of Dent's disease.

Dent's disease, a rare form of proximal tubular dysfunction, is a X-linked recessive disorder with around 250 affected families reported to date. The disease is caused by mutations in either the CLCN5 (Dent disease 1) or OCRL1 (Dent disease 2) genes. CLCN5 encodes the chloride/proton (2Cl−/H+) exchanger ClC-5. The intense endocytic activity in proximal tubule cells involves endosomal acidification that is necessary for dissociation of the ligand-receptor complex and recycling of receptors to the apical membrane. In the apical endosomes, wild-type Cl-/H+ exchanger (figure 6(A)) provides a countercurrent for the proton pump, which facilitates vesicular acidification. In ClC-5 knock-out endosomes (figure 6(B)) positive charges accumulate in the lumen causing defective endocytosis. OCRL1 encodes a member of the family of inositol polyphosphate 5-phosphatases involved in lysosomal trafficking and endosomal sorting. OCRL1 mutations are also seen in Lowe syndrome which in addition to the renal manifestations seen in Dent's disease causes congenital cataracts, mental retardation and muscular hypotonia.(22)

A



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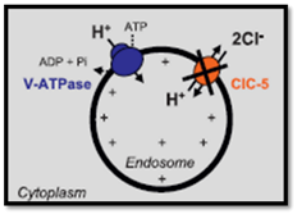

C

Mutations	Effect of mutation
Class 1	ER retention; most common (60%)
Class 2	Delayed protein maturation and reduced stability
Class 3	Normal structure but defective permeation

Figure 6: A. Normal endocytic function in proximal tubule; B. Dent's disease (type 1) with ClC-5 mutation; C. Different types of mutations and corresponding defects seen Dent's disease (type 1)

Defective degradation

Ubiquitylation is the addition of ubiquitin to a substrate protein and acts as a signal for their degradation by the proteasome. Defective ubiquitylation affects the normal degradation process leading to cellular dysfunction as seen in Liddle's syndrome and pseudohypoaldosteronism type 2.(23)

Liddle syndrome is a rare autosomal dominant low renin, volume-expanded hypertension caused by truncating or missense mutations in the epithelial sodium channel β- or γ-subunits. Normally, the E3 ubiquitin ligase NEDD4-2 interacts with PY motif in the ENaC subunits to induce its ubiquitylation and thereby degradation. ENaC mutations interfere with this process resulting in a gain-of-function effect. (24)

Pseudohypoaldosteronism type 2 (PHAII) is a form of autosomal dominant hypertension characterized by increased activity of Na-Cl co-transporter (NCC) in the distal tubule leading to hypertension, reduced distal Na+ delivery and consequent hyperkalaemia. Mutations of KLHL3/CUL3 ubiquitin protein–ligase complex are reported in some cases.(25) NEDD4-2 knockout mice have same phenotypic manifestations as in PHAII and further studies are needed to attribute its role in humans.

Imaging protein trafficking

With better understanding of the molecular basis of disease, the need for real-time imaging of cellular events with high spatial and temporal resolution arises. Genetic mutations are the basis for defective protein trafficking and can be diagnosed by linkage analysis and by use of genome wide association studies. Understanding the consequences of genetic mutations on the protein product are essential steps to understand pathophysiology, improve diagnosis and develop possible therapeutic strategies. This can be achieved by imaging the process of protein trafficking. The spinning disk confocal microscope and total internal reflectance fluorescence (TIRF) microscopy are real time imaging techniques to visualize protein trafficking.(26)

Current and evolving therapies

Understanding the molecular aspects of protein trafficking has led to studies aimed at therapeutic strategies to protect mutants having residual function from apoptosis. Therapies targeting each of the defective steps in protein trafficking are being evaluated in a number of studies (figure 7).

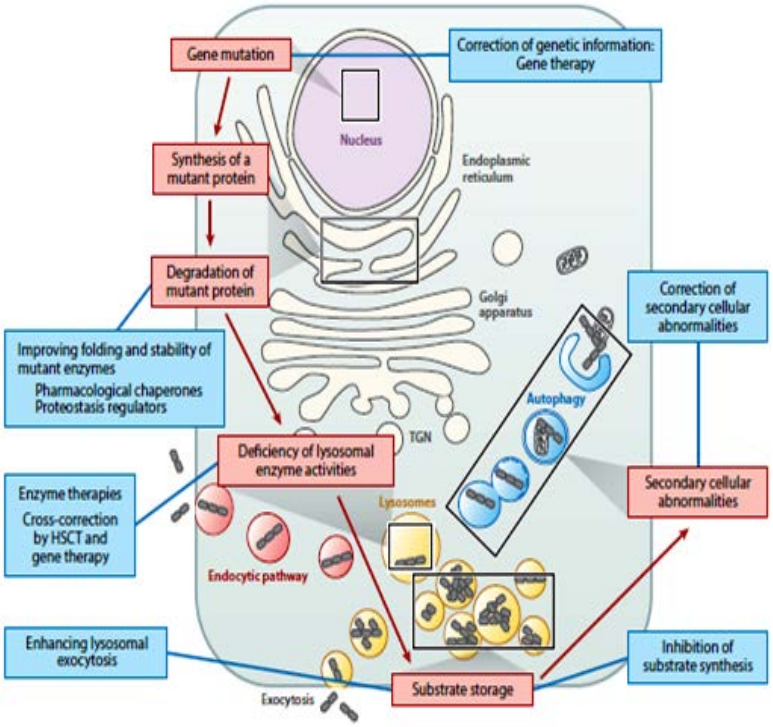

Figure 7: Disorders of protein trafficking and prospective therapeutic strategies

Until now, the idea of manipulating proteostasis was considered viable only for conformational diseases, such as Alzheimer disease or prion disease. Enzyme replacement therapies have become the choice of therapy for lysosomal storage disorders. Molecules that aim to bind and stabilize native state of mutant protein in the folded form are desirable.(27) The different types of small molecules to serve this purpose include

1. Chemical chaperones (28)

Glycerol, deuterated water, trimethylamine N-oxide (TMAO), DMSO and other low molecular weight compounds, have the property of increasing cellular osmolar activity and partially correct the mislocalization of mutant proteins. DMSO has shown promise in restoring AE1 function in distal RTA.(29) Lack of specificity and need for high concentrations lead to the development of pharmacological chaperones.

2. Pharmacological chaperones

Pharmacological chaperones bind directly to misfolded protein with high specificity and stabilize them. But it should be remembered that they are effective for only one type of mutation. Hence, not all cases with the same

disease would respond to a particular pharmacological chaperone. Sapropterin dihydrochloride for phenylketonuria and tafamidis for transthyretin-related hereditary amyloidosis have already been approved for this purpose. SR49059 has some beneficial effects in rescuing AVPR2 mutants in NDI.(30)

3. Proteostasis regulators

Proteostasis regulators serve to augment protein folding, regulate protein synthesis, degradation, aggregation and post-translational modification.(5) In simple words they influence all components of the protein trafficking pathway. The developmental agents, mechanism of action and probable target diseases are shown in table 2.

Agent	Mechanism	Therapeutic use
Protein folding augmentation		
4-PBA	Enhances protein folding	Diabetic nephropathy, CFH defieciency
TUDCA , ORP-150	Enhances protein folding	Fibrosis
Migalastat	Prevents misfolding of mutant galactosidase	Fabry's disease
BIX	Chaperone inducer	-
Protein translation attenuator		
Salubrinal	Cellular eIF-2α phosphatase inhibitor	Hyperglycemia & nephrotoxin induced renal injury
Guanabenz	Stress-induced eIF-2α phosphatase inhibitor	Antihypertensive
Quercetin	Activates IRE1–XBP1 axis	Ischemia reperfusion injury
Modulation of protein degradation		
Bortezomib, Carfilzomib	Inhibits chymotrypsin-like sites of proteasome	Antibody mediated rejection, glomerulonephritis
Modulation of post-translational acetylation		
Sirtuin 1	Attenuates UPR response	Diabetic nephropathy

Table 2: Proteostasis regulators in kidney disease

Conclusions

Protein trafficking plays a major role in the quality control and maintenance of the functional diversity of proteins. Accumulating evidence emphasizes that normal protein trafficking is altered by stress conditions. This strongly contributes to the development and progression of kidney disease. Increasing knowledge of mechanisms modulating protein folding and trafficking has renewed interest in this field. More sophisticated assays for drug discovery and systematically defining the effects of compounds on the pathway are major needs. Finally, predicting and defining the possible side effects of manipulating protein trafficking at the systemic level remains an important subject for the validation of the pathway as a true drug target.

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