PROTEINOPATHIES

Study of ADTKD-*MUC1* identifies a new lead for the treatment of toxic proteinopathies

“ cargo receptors like TMED9 entrap misfolded proteins such as MUC1-fs in the early secretory pathway ”

A new study by Anna Greka and colleagues provides insight into the molecular mechanisms that underlie autosomal dominant tubulointerstitial kidney disease–*MUC1* (ADTKD–*MUC1*; also known as MKD). In addition, the researchers identify a new lead compound for the treatment of this rare disease and potentially other toxic proteinopathies.

ADTKD–*MUC1* is caused by a frameshift mutation in *MUC1*, which results in the production of a mutant mucin 1 neo-protein (MUC1-fs). To investigate the mechanism by which this neo-protein leads to progressive kidney disease, the researchers began by characterizing its cellular localization. In a patient kidney biopsy sample, patient kidney organoids and the kidneys of mice with heterozygous knock-in of human MUC1 or MUC1-fs, the wild-type protein localized to the apical membrane of tubular epithelial cells, whereas MUC1-fs was located in a punctate pattern throughout the cytoplasm.

Misfolded proteins activate the unfolded protein response (UPR), which regulates cellular proteostasis and has both cytoprotective and proapoptotic effects. Greka and colleagues show that accumulation of MUC1-fs in a tubular epithelial cell line generated from a patient with ADTKD–*MUC1* activated the

cytoprotective ATF6 branch of the UPR. As inhibition of ATF6 increased the intracellular accumulation of MUC1-fs, they suggest that ATF6 activation is a protective mechanism that might explain the late onset of kidney failure in this disease. They speculate that disease progression might occur as a result of additional insults such as inflammation and a decline in UPR homeostasis with age.

High content screening and profiling of a library of compounds identified BRD4780 as a promising therapeutic lead for ADTKD–*MUC1*. BRD4780 dose-dependently reduced the level of MUC1-fs, but not of wild-type MUC1, in the patient tubular epithelial cell line. Similarly, BRD4780 selectively removed accumulated MUC1-fs from the kidneys of heterozygous MUC1-fs knock-in mice and from patient-derived kidney organoids. The treatment also downregulated pathways associated with endoplasmic reticulum (ER) stress and the UPR in the mouse model.

Membrane-associated proteins are transported from the ER to the Golgi apparatus in COPII vesicles before being delivered to the apical plasma membrane via the endosomal compartments. Retrograde transport in COPI vesicles may return immature or misfolded proteins from the cis-Golgi to the ER. As MUC1-fs is retained in the cytoplasm, Greka and

colleagues hypothesized that it may be trapped in the vesicles of the early secretory pathway. Using colocalization studies, they show that MUC1-fs is most abundant in the cis-Golgi compartment and in cargo

receptor TMED9-containing vesicles that traffic between the cis-Golgi and the ER. Treatment with BRD4780 reduced the level of TMED9 and enabled trafficking of MUC1-fs along the secretory pathway into lysosomes where it could be degraded.

This effect was phenocopied by deletion of TMED9, and cellular thermal shift assays confirmed that this cargo receptor is a molecular target of BRD4780. The researchers speculate that BRD4780 promotes the anterograde trafficking of MUC1-fs by blocking the binding of TMED9 either to MUC1-fs or to other vesicular proteins.

Finally, the researchers show that BRD4780 can also remove other misfolded membrane-associated proteins that underlie toxic proteinopathies. The compound reduced the levels of mutant uromodulin (UMOD), which causes UMOD-associated nephropathy, and of a mutant rhodopsin protein, which causes retinitis pigmentosa, in cell lines. By contrast, it did not reduce the accumulation of a mutant huntingtin protein that accumulates in the cytoplasm and nucleus in Huntington disease.

“The finding that cargo receptors like TMED9 entrap misfolded proteins such as MUC1-fs in the early secretory pathway is new biology,” comments Greka. “Based on this biological mechanism we have identified a new, molecularly targeted therapeutic strategy that might work in ADTKD–*MUC1*, ADTKD–*UMOD* and several other toxic proteinopathies.”

Ellen F. Carney

ORIGINAL ARTICLE Dvela-Levitt, M. et al. Small molecule targets TMED9 and promotes lysosomal degradation to reverse proteinopathy. *Cell* **178**, 521–535 (2019)