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Autophagy in the proximal tubule cell and its role in the progression of chronic kidney disease

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AUTOPHAGY IN THE PROXIMAL TUBULE CELL AND ITS ROLE IN THE PROGRESSION OF CHRONIC KIDNEY DISEASE

by

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DEDICATION

I would like to dedicate this work to my wonderful parents, Jeffrey Kondrat and Janet Boyle; my amazing and inspiring little brother James and finally my late grandpa John Boyle for inspiring me to be resolute in the pursuit of my goals.
AUTOPHAGY IN THE PROXIMAL TUBULE CELL AND ITS ROLE IN THE PROGRESSION OF CHRONIC KIDNEY DISEASE

JASON RAYMOND KONDRAT

ABSTRACT

Chronic kidney disease is a substantial health problem effecting a large portion of the US population. Presence of excess protein, particularly albumin, in the urine of patients with chronic kidney disease is an independent risk factor for cardiovascular disease and progression to end stage renal disease. In addition, excess protein reabsorption in the proximal tubule is sufficient to cause damage to the proximal tubule independent of the initial condition that lead to chronic disease. In the last decade, excess protein reabsorption by the proximal tubule as a result of chronic kidney damage has been shown to cause oxidative and ER stress, cell death, as well as tubule inflammation and fibrosis in the proximal tubule cell. Only recently have two studies investigated the role of autophagy in protein-induced tubule damage. Autophagy is a dynamic catabolic mechanism used to degrade cytosolic elements in times of cell starvation and is an important process in the cell’s response to stress. The results of the studies by Wei Jin Liu et al. and Yamahara et. al. provide important first steps to determine whether autophagy of excess protein in proteinuric states prevents proximal tubule cell toxicity and potentially slow the progression of chronic kidney disease (CKD). This thesis will explore the results of these two studies in the context of proximal tubule damage in chronic kidney disease, and the discuss the potential for protein autophagy to improve our understanding and treatment of chronic kidney disease.
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Regulatory interactions of PKB, PKC, and the RAS may cause a vicious cycle of ROS production and excess endocytosis of albumin
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<table>
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<th>Abbreviation</th>
<th>Description</th>
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<tbody>
<tr>
<td>ACR</td>
<td>Albumin Creatinine Ratio</td>
</tr>
<tr>
<td>AMPK</td>
<td>AMP Activated Protein Kinase</td>
</tr>
<tr>
<td>ANGII</td>
<td>Angiotensin II</td>
</tr>
<tr>
<td>AT</td>
<td>Angiotensin II receptor</td>
</tr>
<tr>
<td>ATF6</td>
<td>Activating transcription factor 6</td>
</tr>
<tr>
<td>Atg</td>
<td>Autophagy-regulated gene</td>
</tr>
<tr>
<td>ATM</td>
<td>ataxia-telangiectasia mutated protein kinase</td>
</tr>
<tr>
<td>CKD</td>
<td>Chronic Kidney Disease</td>
</tr>
<tr>
<td>CSA</td>
<td>Cyclosporine A</td>
</tr>
<tr>
<td>CVD</td>
<td>Cardiovascular Disease</td>
</tr>
<tr>
<td>DFCP1</td>
<td>Double FYVE-containing protein 1</td>
</tr>
<tr>
<td>EGF</td>
<td>Epidermal Derived Growth Factor</td>
</tr>
<tr>
<td>ER</td>
<td>Endoplasmic Reticulum</td>
</tr>
<tr>
<td>ERK</td>
<td>extracellular-signal-regulated kinase</td>
</tr>
<tr>
<td>ESRD</td>
<td>End Stage Renal Disease</td>
</tr>
<tr>
<td>ETF</td>
<td>Electron Transfer Flavoprotein</td>
</tr>
<tr>
<td>ETF/ETF coQ</td>
<td>flavoprotein (ETF) and ETF-coenzyme Q oxidoreductase</td>
</tr>
<tr>
<td>FFA</td>
<td>Free Fatty Acid</td>
</tr>
<tr>
<td>GFP</td>
<td>Green Fluorescent Protein</td>
</tr>
<tr>
<td>HK-2</td>
<td>Human Immortalized Kidney Cell Line</td>
</tr>
<tr>
<td>HAVCR1</td>
<td>Hepatitis A virus cellular receptor 1</td>
</tr>
<tr>
<td>Abbreviation</td>
<td>Description</td>
</tr>
<tr>
<td>--------------</td>
<td>-------------</td>
</tr>
<tr>
<td>HFD</td>
<td>High Fat Diet</td>
</tr>
<tr>
<td>HAS</td>
<td>Human Serum Albumin</td>
</tr>
<tr>
<td>IGFR</td>
<td>Insulin-like Growth Factor Receptor</td>
</tr>
<tr>
<td>IgG</td>
<td>Immunoglobulin G</td>
</tr>
<tr>
<td>IL-8</td>
<td>Interleukin-8</td>
</tr>
<tr>
<td>IKK</td>
<td>Inhibitor of Kappa B</td>
</tr>
<tr>
<td>IKK</td>
<td>IκB kinase</td>
</tr>
<tr>
<td>IRE1</td>
<td>Serine/threonine-protein kinase/endoribonuclease</td>
</tr>
<tr>
<td>JNK1</td>
<td>c-Jun N-Terminal Kinase 1</td>
</tr>
<tr>
<td>K/DOQI</td>
<td>The Kidney Disease Outcomes Quality Initiative</td>
</tr>
<tr>
<td>KDIGO</td>
<td>Kidney Disease: Improving Global Outcomes Organization</td>
</tr>
<tr>
<td>LAMP</td>
<td>Lysosome-associated membrane protein</td>
</tr>
<tr>
<td>LC3</td>
<td>Microtubule-associated protein 1A/1B-light chain 3</td>
</tr>
<tr>
<td>LCN2</td>
<td>Lipocalin-2</td>
</tr>
<tr>
<td>L-FABP</td>
<td>liver-type fatty acid binding protein</td>
</tr>
<tr>
<td>LK-B1</td>
<td>liver kinase B1</td>
</tr>
<tr>
<td>MCA</td>
<td>Megalin, Cubilin, Amnionless</td>
</tr>
<tr>
<td>MCP1</td>
<td>Monocyte Chemotactic Protein-1</td>
</tr>
<tr>
<td>MCNS</td>
<td>Minimal Change Nephrotic Syndrome</td>
</tr>
<tr>
<td>MEK</td>
<td>Mitogen Activated Protein Kinase</td>
</tr>
<tr>
<td>MG132</td>
<td>N-benzyloxycarbonyl (Cbz)-Leu-Leu-leucinal</td>
</tr>
<tr>
<td>mTOR</td>
<td>Mammalian Target of Rapamycin</td>
</tr>
</tbody>
</table>
MTT ......................................... 3-[4,5-dimethylthiazol-2-yl]-2,5 diphenyl tetrazolium bromide
ND ................................................................................................. Normal Diet
NF-κB ....................... Nuclear Factor Kappa-Light-Chain-Enhancer of Activated B Cells
PARP ................................................................. poly ADP ribose polymerase
PBS .............................................................. Phosphate Buffered Saline
PERK .......................................................... PKR-like ER Kinase
PI3K .......................................................... phosphatidylinositol 3-kinase
PKC .......................................................... Protein Kinase C
PTC ............................................................. Proximal Tubule Cell
RANTES .................. Regulated on Activation, Normal T cell Expressed and Secreted
ULK ........................................................... Atg 12/unc-51-like Kinase
RAS ......................................................... Renin Angiotensin System
ROS ............................................................ Reactive Oxygen Species
RHEB ........................................................ Ras homolog enriched in brain
SQSMT1 ...................................................... Sequestosome 1
TEM .......................................................... Transmission Electron Microscopy
TR ............................................................. Texas Red
TUDCA ........................................................ Taurourodeoxycholic acid
TSC ............................................................ Tuberous Sclerosis Complex
UPS ............................................................ Ubiquitin Proteosome System
WIPi .................................................... WD repeat domain phosphoinositide-interacting protein
INTRODUCTION

CHRONIC KIDNEY DISEASE

Chronic kidney disease (CKD) is the presence of persistent kidney abnormalities last for ≥3 months manifest as a decrease in eGFR below <60 ml/min/1.73 m², and/or the presence of markers of kidney damage including proteinuria, histological/structural abnormalities, urine sediment abnormalities, and an electrolyte imbalance due to tubule dysfunction.¹ The Kidney Disease Outcomes Quality Initiative (K/DOQI) first staged CKD primarily on the basis of eGFR, with stages 1 and 2 above 60 ml/min/1.73 m² requiring additional evidence of kidney damage, such as proteinuria, in order to diagnose a patient with CKD.¹ If the decline in GFR or damage to kidney function becomes so severe that renal function requires assistance from dialysis or a kidney transplant, then the patient is no longer said to have CKD but has progressed to End Stage Renal Disease (ESRD).²

Although the K/DOQI is still the most commonly used staging of CKD among research and clinicians, in the 10 years since the K/DOQI, other initiatives such as the Kidney Disease: Improving Global Outcomes Organization (KDIGO) have attempted to more fully integrate proteinuria in the definition and staging of CKD.³ This was partly in response to mounting evidence that a patient with a given GFR more rapidly progresses to ESRD with concomitant proteinuria.³,⁵-⁹
Table 1: The K/DOQI staging of Chronic Kidney disease

<table>
<thead>
<tr>
<th>STAGE</th>
<th>DESCRIPTION</th>
<th>GFR (ml/min/1.73m²)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>At increased risk</td>
<td>≥90</td>
</tr>
<tr>
<td>1</td>
<td>Kidney damage with normal or ↑ GFR</td>
<td>≥90</td>
</tr>
<tr>
<td>2</td>
<td>Kidney damage with mild ↓ GFR</td>
<td>60-89</td>
</tr>
<tr>
<td>3</td>
<td>Moderate ↓ GFR</td>
<td>30-59</td>
</tr>
<tr>
<td>4</td>
<td>Sever ↓ GFR</td>
<td>15-29</td>
</tr>
<tr>
<td>5</td>
<td>Kidney failure</td>
<td>&lt;15 (or dialysis)</td>
</tr>
</tbody>
</table>

Patients with CKD stages below 60 ml/min/1.73 m² have an increased risk of cardiovascular disease (CVD) and cardiovascular-related death.⁴,¹⁰ According to the KDIGO:

“People with CKD are more likely to experience a cardiovascular event than to progress to ESRD, have a worse prognosis with higher mortality after acute myocardial infarction, and have a higher risk of recurrent MI, heart failure and sudden cardiac death.”⁸

In fact, nearly half of patients with a GFR below 60ml/min/1.73m² die from cardiovascular related events. In addition, the percentage of patients with CVD related deaths increases as the CKD stage advances.¹¹,¹² That is, a patient with stage 5 CKD has a greater chance of dying from CVD than a patient in stage 3. Of the surviving patients, many progress to ESRD. Patients with ESRD on dialysis have a life expectancy of 5 years in the absence of a kidney transplant.¹¹,¹² The presence of proteinuria not only
promotes the progression of chronic kidney disease towards ESRD, but independently increases the risk of CVD mortality.\textsuperscript{3,7,12}

CKD is a significant public health issue effecting millions of Americans each year and increasingly straining the US healthcare system. According to the 2012 National Health and Nutrition Examination Survey conducted by the United States Renal Data System, from 2005-2010, 6.3\% of patients in the US were diagnosed with CKD when it was defined by an eGFR<60 ml/min/1.73 m\(^2\).\textsuperscript{1,11} However; when CKD was defined by an Albumin Creatinine Ratio (ACR), a quantitative measure of proteinuria, greater than 30 mg/g the percent of subjects diagnosed with CKD increased to 9.2\%.\textsuperscript{1} If defined by ACR, the prevalence of CKD rivals other major health problems including diabetes (8.5\%) and cardiovascular disease (9.3\%).\textsuperscript{1} Properly classifying chronic kidney disease is essential to determine the scope of this disease and how to allocate research and funding for treatment. Understanding proteinuria’s effectiveness as a prognostic indicator of CKD and more importantly how it affects the progression of CKD is paramount in the future classification and treatment of CKD.
**PROTEINURIA**

Large blood proteins (>60kDa) such as albumin and immunoglobulins are too large to pass through the glomerular filtration barrier of the kidney and are present in urine in only small amounts. Light weight molecular proteins such as β2 and α1 microglobulins, lysozyme, and Vitamin D binding protein pass through the filtration barrier and are normally present in the glomerular filtrate at a concentration approximately .072 mg/ml. Of the proteins that are filtered at the glomerulus, 95%-97% are reabsorbed in the proximal tubule. These proteins are usually endocytozed, degraded, and their amino acids re-circulated. As a result, urine protein content is very low (30-130mg/24hrs) and “consists primarily of plasma albumin (40%), immunoglobulin fragments (15%), and other proteins (5%).” Proteinuria is classically defined as excess protein in urine exceeding 150mg/24hrs or a urine protein concentration of 10mg/dl. These threshold numbers are not fixed and current evidence suggests progressive renal disease and CVD are associated with even smaller amounts of proteinuria.

Albumin is the major protein driving the plasma’s osmotic pressure gradient. It is also a carrier protein for various substances such as fatty acids, bilirubin, vitamins, and hormones. Albumin is an especially important protein in CKD because it is the most abundant plasma protein (normally 5g/dl). Due to its size and negative charge, albumin is not normally filtered by the glomerulus and is usually present in the urine in a concentration of only 0.0229 mg/ml. An ACR of <30mg of albumin/g creatinine is
considered within a normal physiologic range. Increasing ACR above 30mg/g Cr increases the risk of kidney damage and CVD.\textsuperscript{16} According to the KDIGO:

“Albumin is the principle component of urinary protein in most kidney disease and epidemiological data from studies around the world demonstrate a strong, graded relationship between the quantity of urine albumin with both kidney and CVD risk.”\textsuperscript{11}

In fact, excess excretion of albumin, or albuminuria, is the most frequent type of proteinuria and carries the greatest risk factors for development of CVD and cardiovascular dysfunction.\textsuperscript{4,17,18}

**Figure 1: Schematic of Proximal Tubule in Proteinuric Kidney Disease; (Left) Normal handling of protein; (Right) Pathologic excess of filtered protein leading to proteinuria.\textsuperscript{16}**

There are three major types of proteinuria: glomerular, tubular and overflow.\textsuperscript{19} Glomerular proteinuria is the most common type of proteinuria. It is the only type capable of generating protein excretions greater than (4g/24hrs), and thus is the most relevant type of proteinuria in CKD.\textsuperscript{19} Glomerular proteinuria results from damage and
dysfunction to the glomerulus that increases permeability of the normal glomerular filtration barrier causing protein to accumulate in the filtrate.\textsuperscript{19} Persistent glomerular proteinuria often results in nephrotic syndrome. Nephrotic syndrome is characterized by persistent albuminuria, hypoalbuminemia, hyperlipidemia, and lipiuria. Albuminuria and lipiduria are the result of increased glomerular filtration of these substances due to damage to the glomerular filtration barrier.\textsuperscript{19,20} Excessive excretion of albumin, as well as increased degradation of albumin after absorption in the proximal tubule, lead to hypoalbuminemia that in turn creates a decreased osmotic pressure gradient in the capillaries and increased tubular sodium reabsorption that causes edema.\textsuperscript{20,21}

Focal segmental glomerulonephritis/glomerular sclerosis has a prevalence of 4% and is the “most common primary glomerular disorder causing end-stage renal disease in the United States.”\textsuperscript{22,23} Although there are many causes of focal segmental glomerulonephritis, including genetic and pharmacological risk factors, the most common cause is idiopathic.\textsuperscript{23}

Minimal change disease is another cause of proteinuria and is the most common cause of primary glomerular proteinuria in children with nephrotic syndrome.\textsuperscript{24} Other causes of primary glomerulonephritis include membranous nephropathy, in which antibody deposition and inflammation is seen in the glomerulus, and membranoproliferative glomerulonephritis, characterized by thickening of the glomerular basement membrane.\textsuperscript{20} Common causes of secondary glomerular nephropathies are diabetes; infection from HIV, hepatitis B and C, malaria, or endocarditis; lupus nephritis,
an autoimmune kidney disease resulting from systemic lupus erythematosus; chronic transplantation rejection; drug/toxin induced nephropathies from agents including NSAID’s, heroin, gold, and heavy metals.²⁰

Tubular proteinuria is the result of damage and dysfunction of the proximal tubule that decreases reabsorption of low molecular weight proteins resulting in their excretion. The most common causes of tubular proteinuria result from dysfunction of the tubulointerstitium.⁴,¹⁹ Hypertension damages the glomerulus and tubulointerstium resulting in hypertensive nephrosclerosis.⁴ Hypertension, unless malignant, produces only minor levels of tubular proteinuria and does not cause nephrotic syndrome.¹⁹,²⁰ Other causes of tubular proteinuria include Fanconi syndrome, heavy metal toxicity, sickle cell disease, polycystic kidney disease, and uric acid nephropathy.¹⁹

Lastly, overflow proteinuria results from an overproduction of certain plasma proteins causing over filtration in the glomerulus. Excess production of immunoglobulin, usually because of underlying multiple myeloma, is the most common cause of overflow proteinuria.¹⁹,²⁰ Other causes include hemoglobinemia, myoglobinemia, and amyloidosis.¹⁹,²⁰ Due to the fact that the glomerular filtration barrier remains largely intact, overflow proteinuria produces the least proteinuria and is not considered to cause CKD.¹⁹
**THE CORE MAMMALIAN AUTOPHAGIC MECHANISM**

Autophagy is a highly regulated and complex process that permits the cell to engulf large amounts of its own cytoplasmic components, such as abnormal protein aggregates or damaged organelles, into structures called autophagosomes. These autophagosomes fuse with lysosomes, creating an autophagolysosome, that internally degrade cytoplasmic components.25-28 There are three forms of autophagy: macroautophagy, microautophagy, and chaperone-mediated autophagy. Macroautophagy is most relevant to the study of autophagy in response to cellular stress.28,29 The core autophagic mechanism involve 4 major components:

“(1) mTOR and the Atg1/unc-51-like kinase (ULK) complex; (2) the Beclin 1/class III phosphatidylinositol 3-kinase (PI3K) complex; (3) two ubiquitin-like protein (Atg12 and Atg8/LC3) conjugation systems; and (4) proteins that mediate fusion between autophagosomes and lysosomes such as LAMP2.”28

This core autophagy cellular mechanism is ubiquitous throughout all eukaryotes.26

Figure 2: The Core Mammalian Autophagic Pathway 28
The mTORC1 complex regulates cell growth as well as autophagy and plays an important role in sensing the cells nutrient levels (particularly amino acids). In nutrient replete conditions, mTORC1 is constitutively bound to the ULK complex. The kinase activity of mTORC1 phosphorylates the ULK1 on Serine 757 inhibiting the ULK complex’s ability to activate autophagy. **During amino acid deficiency**, mTORC1 is phosphorylated in the causing it to disassociate from the ULK complex. Once it is disassociated from mTORC1, ULK 1 is dephosphorylated and is free to induce autophagy.\(^{28,29,30}\) The ULK complex is largely responsible for the induction of autophagy under conditions of nutrient and energy starvation.

Another important **autophagy** regulator is AMP activated protein kinase (AMPK). This enzyme measures energy depletion by sensing the cellular AMP:ATP ratio.\(^{28,31}\) Apart from sensing energy depletion, AMPK is also integral in cell’s response to oxidative and inflammatory stress.\(^{31}\) The most commonly proposed mechanism suggests that AMPK activates autophagy by inhibiting mTOR via phosphorylation of the regulatory proteins tuberous sclerosis complex 2 (TCS2) and Raptor. In addition, AMPK can also activate autophagy independent of mTOR through phosphorylation of the serine 317 and serine 777 residues of ULK 1.\(^{28,31}\)
Figure 3: AMPK activates autophagy through inhibition of mTOR and activation of ULK1/2

The Beclin 1/class III phosphatidylinositol 3-kinase complex produces phosphoinositol-3-phosphate that in turn interacts with DFCP1 and WIPI. This process extracts lipids from various intracellular sites around the cell including mitochondria, endoplasmic reticulum, and plasma membrane.\textsuperscript{25,26,28} The nucleation of these lipids form a primitive omegosome that matures into a phagophore.\textsuperscript{25,26,28} Beclin-1 plays an important role in stress-induced activation of autophagy and links autophagy to apoptosis. Normally, Beclin-1 is bound and inactivated by bcl-2. Specific cellular stresses activate kinases such as c-Jun N-terminal kinase-1 (JNK1), death-associated protein kinase, and nuclear factor kappa-light-chain-enhancer of activated B cells (NF-κB) that disassociate bcl-2 and Beclin-1.\textsuperscript{28}
Figure 4: mTOR and Beclin-1 as upstream regulators of autophagy: (LEFT) mTOR inhibition induces autophagy through ULK1/2 (RIGHT) Beclin 1/class III phosphatidylinositol 3-kinase complex reacts to certain stresses, in particular ER stress, and induced autophagy.  

The LC3 and Atg12 ubiquiton-like conjugation systems are involved in the elongation and closure of the phagophore, resulting in autophagosome formation. LC3-I is the cytosolic form of LC3 and upon lipid conjugation through Atg, forms LC3-II. LC3-II is then incorporated into the double membrane of the growing phagophore. LC3-II is an important protein in determining the extent of autophagosome formation and is classically used as a marker of autophagy in biochemical analyses.  

Interestingly, several LC3-II association proteins bind ubiquinated proteins and mitochondria, causing selective degradation of certain cytoplasmic elements. One such protein p62, also known as SQSMT1, associates with LC3-II on the autophagosome inner membrane. The p62/LC3-II complex then binds ubiquinated proteins targeted for destruction. Thus, p62 serves as a bridge between LC3-II and ubiquinated protein aggregates.  

After maturation, the autophagosomes fuse with lysosomes to create an autophagolysosome. Inside the autophagolysosome, proteases degrade p62. Analysis of SQSMT1 is used as a measure of autophagic flux. Cytosolic levels of p62 decrease
as p62 is degraded in the autophagosome. Therefore, the more p62 expression is suppressed, the faster autophagolysosomes are being formed, thereby increasing autophagic flux.\textsuperscript{32}

Once the phagophore encloses and engulfs the necessary cytoplasmic elements to form an autophagosome, it must fuse with a lysosome to make an autophagolysosome in order to break down various cytoplasmic contents.\textsuperscript{28} Lysosomal associated membrane proteins 1 and 2 (LAMP 1-2) make up 50\% of lysosomal membrane proteins. LAMP 1-2 are integral for proper lysosomal fusion with the autophagosome and also assist in the proper transport of lysosomes to the autophagosomes.\textsuperscript{28}

Autophagy functions as a survival response to maintain nutrient and energy homeostasis in times of starvation. It also degrades aggregate proteins and dysfunctional mitochondria. Autophagy is a self-limiting process that protects the cell from stress. If the stress is counteracted, then the signal that induced autophagy is terminated and the cell survives. If the stress cannot be counteracted by autophagy, the cell undergoes apoptosis. Many of the same enzymes that induce apoptosis, most notably caspase-3, also inhibit autophagy. Therefore, even if the cell is unable to successfully counteract the stressor, autophagy is a self-limiting process.

\textit{Pathophysiology of Proteinuria in the Proximal Tubule}

Due to the positive correlation between proteinuria and CKD, there has been extensive research into pathogenesis and consequences of proteinuria. Damage from proteinuric kidney disease is most evident in the glomerulus and the proximal tubule.\textsuperscript{4}
Glomerular dysfunction is the most common cause of CKD and thus damage due to protein overload in the glomerular filtration barrier is always in conjunction with damage from another underlying condition.\textsuperscript{19,20} Excess protein in the proximal tubule is sufficient to cause major damage and dysfunction independent of the initial glomerular injury. This makes the proximal tubule an effective target of research into the pathologic effect of proteinuria in patients with CKD.\textsuperscript{35} A substantial amount of research has been conducted on excess protein in the proximal tubule however, the particular molecular mechanisms causing the detrimental effects of protein overload in the kidney are not completely understood. In the past few years however, a broad outline of the pathophysiology of proteinuria in the proximal tubule has emerged.

A complex comprised of megalin, cubilin, and amnionless (MCA) mediates proximal tubule endocytosis of proteins and other macromolecules. In proteinuric kidney disease, the influx of protein results in a strain on the increases the demand for proximal tubule reabsorption.\textsuperscript{43,52} As a result, any proteins that are unable to be reabsorbed and are excreted in the urine.\textsuperscript{15} The consequence of saturating the reabsorptive process results in an excess of protein containing endosomes inside the proximal tubule.\textsuperscript{15} Furthermore, due to glomerular damage, many proteins rarely filtered or not filtered to a great extent, are seen in the contents of endosomes after reabsorption. The content of endosomes in proteinuric kidney diseases contain high concentrations of free fatty acid bound albumin, various immunoglobulin’s, transferrin, and growth factors that would not be seen in high concentrations in endosomes of a normally functioning kidney.\textsuperscript{15}
Figure 5: Excess reabsorption in the PTC during CKD (a) Megalin/cubilin/amnionless complex regulation of endocytosis during normal physiological conditions; (b) Megalin/cubilin/amnionless complex regulation of endocytosis due proteinuria. The non-specific endocytosis in a proteinuric state causes endocytosis of protein and macromolecules not normally seen in vesicles inside the proximal tubules.  

Excess albumin in the filtrate is particularly detrimental to patients with CKD. However, excess reabsorption of albumin itself does not appear to be significantly detrimental to the PTC. In fact, lipid moieties bound to albumin cause the major pathologic features in the PTC. Research shows that exposure of PTC to delipidated pure albumin at concentrations detected in nephrotic syndrome are handled quite well in normal functioning PTCs. The free-fatty acids bound to albumin are directly linked to oxidative stress by causing mitochondrial dysfunction and generation of reactive oxygen species (ROS). It should be noted that although FFA attached to albumin cause significant pathology, other factors could also generate ROS and damage the PTC. Excess ROS in the PTC can cause DNA damage; protein misfolding leading to ER stress; as well as production of cytokines including TGF-β, MCP-1, RANTEs and IL-
These cytokines promote fibrosis, inflammation, and tubulointerstitial injury. Cells undergoing oxidative, inflammatory, and ER stress are likely to undergo autophagy to neutralize the effect of these stresses. Although it is not known which stress; (oxidative, inflammatory, or ER); is most detrimental to the PTC, the result is apoptosis, atrophy, and fibrosis to the surrounding interstitium.

**FIGURE 6**: A summary of the mechanism of protein-induced cell death in the proximal tubule: Reabsorption of the FFA bound to albumin causes oxidative stress that promotes inflammation, ER stress, autophagy and cell death. Autophagy inhibits the apoptosis activated by oxidative stress, ER stress, and tubulointerstitial injury.

Originally thought as a catabolic pathway activated in times of low energy and low nutrients, autophagy is an important adaptive mechanism for maintaining the viability of many cells in response to cellular stress. Despite these findings, the role of autophagy in proteinuric kidney disease remains a largely understudied field. Only two studies have assessed the role of autophagy in PTC in response to increased protein
load. This thesis will look at these two studies that link proteinuria to autophagy and will place the major findings of these studies in context with the known untoward consequences of proteinuria in the PTC.
PUBLISHED DATA ON AUTOPHAGY IN THE PROXIMAL TUBULE IN PROTEINURIC KIDNEY DISEASE

AUTOPHAGY ACTIVATION INDUCES RENAL TUBULAR INJURY INDUCED BY URINIARY PROTEINS WEI JING LIU ET AL.

The study conducted by Wei Jing Liu et al. working at both the Guandong Medical College and the University of Kansas Medical Center, exposed immortalized HK-2 cells to proteins extracted from the urine of patients with Minimal Change Nephrotic Syndrome (MCNS) to determine whether the autophagic pathway is activated in response to protein overload. As an in vivo model, immunoflorescent and electron microscopy technology was used to examine PTC’s in patients with Minimal Change Nephrotic Syndrome. 27

Immunoflorescent examination of PTC showed significant increase in LC3-II positive immunoflorescent “dots” as compared to control. This was supported by observation using a transmission electron microscopy (TEM). TEM showed an increase in the number of both autophagosomes and autophagolysosomes. 27
Figure 7: Quantitative analysis of autophagy in proximal tubules of patients with MCNS. (A and B) immunofluorescent staining of LC3-II in patients with MCNS compared with control. C) TEM images of autophagic vacuoles in patients with MCNS (AP= autophagosome, AL= Autophagolysosome.)

Similar findings were seen in immunoflorescent in vitro analysis. When HK-2 cells were exposed to 0-8 mg/ml of urinary proteins extracted from MCNS patients, slight increases in LC3-II positive dots were observed in PTC’s when exposed to 0.5-1 mg/ml. significantly more LC3-II puncta were observed at protein concentrations of 2-8mg/ml. The appearance of autophagosomes showed time dependence. Eight mg/ml protein exposure showed peak LC3-II dots at 8 hours before declining.

Western blot analysis of lysates harvested for cells exposed to purified albumin showed that LC3-II increased in response to increasing urinary protein concentrations. A Western blot of SQSTM1 demonstrated that elevated LC3-II expression was due to an increase in autophagic flux itself and not from inhibition of downstream LC3-II processing. Moreover, exposure of cells to MCNS urinary protein increased autophagy more than did exposure to normal human serum albumin. This finding suggests that the composition of excess protein being filtered is important in the pathology of proteinuric kidney disease. Other studies have also investigated the
composition of urinary protein on cellular stress in the proximal tubule. In obesity and diabetes mellitus, excess free fatty acids are bound to albumin. After endocytosis by PTC, free fatty acid rich-albumin promoted oxidative stress, compromised mitochondrial dysfunction and upregulated apoptotic factors. 

Figure 8: Analysis of HK-2 cells exposed to MCNS urinary proteins and albumin. (D) Western blot analysis shows that LC3-II expression increases in a dose-dependent manner when exposed to increasing concentrations of proteins extracted from the urine of patients with MCNS. (E) No significant dose-dependent increase in LC3-II was seen when cells were exposed to increasing concentrations of human serum albumin (HSA). (B) Immunofluorescent in vitro analysis of HK-2 cells exposed to either HSA (8mg/ml) or urinary proteins (8mg/ml) extracted from MCNS patients. (F) Western blot analysis of HK-2 cell lysates at varying time points show that peak LC3-II expression at 8 hours over a 24 hour time period.

Lastly, Immunofluorescent and western blot analysis of LAMP1/2 was used to test the effect of exogenous MCNS protein exposure on lysosomal function. Western blot analysis showed no appreciable increase in LAMP2 expression in HK-2 cells exposed to exogenous MCNS protein when compared to control. This indicates that lysosome number is not changed in response to excess MCNS protein. Immunofluorescent staining of LAMP1 or 2 showed an increase in perinuclear clustering of lysosomes in response to urinary protein, that peaked at 8 hours after exposure to exogenous MCNS proteins. Subsequently, this clustering decreased and disappeared at
16hrs and coincided with a decrease in LC3-II. Perinuclear lysosomal clustering allows for more efficient fusion of lysosomes with autophagosomes. The clustering of LAMP 1/2 positive dots around the nucleus indicates an increase in autophagic flux in the HK-2 cells. In summary, HK-2 cell exposure to exogenous MCNS proteins causes no change in the number of lysosomes, but does causes lysosome perinuclear relocation to facilitate better fusion with the autophagosomes.

Using flow cytometry, Wei Jing Liu et al. showed that the production of reactive oxygen species increased in a dose-dependent manner with urinary protein exposure. Furthermore, administration of the antioxidants catalase, a naturally occurring antioxidant that catalyzes the conversion of H2O2 to oxygen and water, and tiron, a iron chelating agent shown to have ROS scavenging properties, significantly reduced LC3-II positive dots and LC3-II expression in HK-2 cells exposed to urinary proteins. Another study also showed that reduction of ROS production was necessary for inactivating Atg4, an essential step in the activation of autophagy in eukaryotic cells. This indicates that excess protein mediated autophagy is a consequence of protein-induced ROS production in the proximal tubule cells.

Although originally proposed to be two independent pathways, there is now evidence linking autophagy and the ubiquitin proteasome system (UBS) involved in ER stress. It was shown that ROS production causes proteasome inhibition and exacerbates ER stress. Proteasome inhibition was shown to result in a compensatory induction of autophagy. In the Wei Jing Lui study, the proteasome inhibitor MG132 increased LC3-II expression in the proximal tubule. However, exposure of HK-2 cells to MCNS proteins
failed to inhibit proteasomes or did the MCNS proteins enhance the inhibitory effect of MG132.”

This suggests that ER stress is unlikely to be involved in protein-induced autophagy in the PTC.

Figure 9: (C-D) Immunofluorescent microscopy comparing LC3-II expression of HK-2 when exposed to MCNS proteins w/ the antioxidants tiron or catalase. (E) Western blot analysis of the same parameters as in C-D.

Wei Jing Lui et al. sought to assess the effect that autophagy had on the viability of the HK-2 cells. To test the effect of autophagy on cell viability, rapamycin, a potent inhibitor of mTOR, was used to increase autophagy or chloroquine, an inhibitor of autophagolysosome degradation, was used to decrease it. In addition, siRNA’s were used to knock down beclin-1 expression in order to down regulate autophagy.

HK-2 exposure to urinary proteins resulted in increased apoptosis, secretion of the renal tubule injury markers LCN2 and HAVCR1, and inhibited cell viability. These effects were enhanced by chloroquine and inhibited by rapamycin. Increased apoptosis,
LCN2 and HAVCR1 expression, and a decrease in cell viability was also seen in cells with si-RNA mediated beclin-1 deficiency. These results indicate that induction of autophagy is likely to be cytoprotective by preventing cell apoptotic cell death.²⁷

Wei Jing Lui et. al’s study demonstrated that protein-induced autophagy in PTC required the generation of ROS. Excess protein reabsorption in the PTC caused mitochondrial dysfunction and subsequent ROS production.³⁹,⁴² The generation of ROS was needed to induce autophagy as well as cause damage to the PTC. Activation of autophagy was able to improve the viability of the PTCs exposed to excess protein indicating that activation of autophagy is used to protect the cell from damage resulting from protein-induced ROS generation.
OBESITY-MEDIATED AUTOPHAGY INSUFFICIENCY EXACERBATES PROTEINURIA-INDUCED TUBULOINTERSTITIAL LESIONS

Obesity is an independent risk factor for tubular damage but the mechanism underlying this relationship is uncertain. Yamahara et al. published a study in December 2013 to examine if the enhanced proximal tubule vulnerability to protein in obese patients was the result of obesity-induced inhibition of autophagy. This investigation required an examination of autophagy in the PTC in response to protein load. Due to its central role in both obesity and PTC damage, FFA-rich albumin was used to inspect autophagy in the PTC.

Mice in the Yamahara et al. study were fed either a normal (ND) or high fat diet (HFD) and received daily intraperitoneal injections of either PBS (control), FFA-replete albumin, or FFA-depleted albumin for 11 days. Renal histology showed that ND mice injected with excess FFA-bound albumin demonstrated prototypical signs of proximal tubule damage including tubular cell flattening, tubular cell vacuolization, and luminal dilation. HFD mice with excess FFA-replete albumin showed increased signs of tubule damage as compared to ND mice. In addition, apoptosis was significantly increased in HFD mice injected with FFA-bound albumin as indicated by an greater cleaved caspase 3 content in a Western blot. Thus, administration of excess FFA-bound albumin in the intact animal is sufficient to cause damage to the proximal tubule. Furthermore, proximal tubule injury is exacerbated by obesity.
Figure 11: Assessment of damage to the proximal tubule in mice on a ND or HFD after 11 days of intraperitoneal FFA-albumin injection. (F) H&E staining of mouse proximal tubule injected with FFA-albumin or PBS with a ND or HFD; (G) damage score determined by F; (H) Quantitative analysis of neutrophil gelatinase-associated lipocalin; (I-J) Western blot analysis of cleaved caspase-3.

To determine whether or not FFA-rich albumin induced autophagy, immunofluorescence microscopy was used on samples of (GFP)-LC3-II expressing transgenic mice. Mice fed ad libitum did not show any increase in (GFP)-LC3-II expression in the kidney. In contrast, mice forced to fast for 48 hours showed increases in (GFP)-LC3-II accumulation in proximal tubules as well as other tissues including skeletal muscle and liver. Mice fed ND showed increased (GFP)-LC3-II in proximal tubules only after daily injection of FFA-albumin, whereas FA-depleted albumin injected mice on a ND did not increase (GFP-LC3II) expression. Furthermore, mice injected with Texas Red (TR) labeled albumin showed increased expression LC3-II in TR positive but not TR negative cells. The FFA-albumin mediated increase in LC3-II expression was enhanced when mice where fed a HFD. LC3-II was no longer enhanced when mice where taken off from the HFD after 4 weeks and put on a ND for 4 weeks. Taken together, this suggests that an increased reabsorption of FFA and not albumin itself is the
major factor responsible for inducing LC3-II expression in the proximal tubule. Furthermore, a HFD inhibited FFA-albumin activation of autophagy and this inhibition is reversible through diet. This conclusion is consistent with prior observations in HK-2 cells, that FFA associated with albumin increases ROS generation and mitochondrial dysfunction in PTC’s obtained from subjects with proteinuric kidney disease. 

Figure 12: Immunofluorescence and Western blot analysis of LC3-II expression in the Yamahara et al study. (A) Immunofluorescence analysis of LC3-II expression in various organs in mice fed ad-labium, fasted for 48 hours, or injected with FFA-rich albumin; (B) Immunofluorescence analysis of LC3-II expression and Texas Red-labeled albumin in FFA-overloaded proximal tubules; (G) Immunofluorescence analysis of LC3-II express in mice proximal tubule in FFA-albumin injected mice compared to control; (H-I) Western blot analysis of parameters depicted in panel G.
Similar to a prior study, Yamahara et. al., investigated the effect of protein induced ER stress on autophagy. In the later study, FFA palmitate exerted-cytotoxic effects on PTC. Yamahara showed that palmitate bound-albumin caused ER stress by demonstrating that it increased the expression of phosphorylated PKR-like ER kinase (PERK). These investigators then showed that reducing ER stress with taurourodeoxycholic acid (TUDCA) did not effect palmitate-bound albumin-induced LC3-II expression. The association between ER stress and autophagy was further invalidated by an immunofluorescence study wherein TUDCA was ineffective at reducing GFP-LC3-II expression in the proximal tubules of protein overloaded transgenic mice.

After determining that FFA-rich albumin induced autophagy, Yamahara et. al., attempted to identify whether or not two major autophagic regulatory signals, AMPK or mTOR, where responsible for FFA induced autophagy. siRNA’s for either TSC1, an inhibitor of mTOR, or AMPKα, an AMPK subunit that when phosphorylated activates AMPK, where used to activate mTOR or inhibit AMPK, respectively. Yamahara et. al., showed that both siRNA directed against TSC1 and AMPKα decreased autophagy caused by palmitate-bound albumin. Western blot analysis showed that palmitate-bound albumin did not significantly affect P70S6k and S6 proteins; downstream effectors of mTOR commonly used to estimate mTOR activity. In contrast, Western blot analysis showed that palmitate-bound albumin increased the phosphorylation of AMPKα. These results suggest that AMPK activation is a consequence of FFA excess and AMPK activation is needed to activate autophagy. In addition, mTOR inhibition is not the likely mechanism in which FFA excess induced autophagy in the proximal tubule.
In contrast to FFA-rich albumin induced autophagy, activated AMPKα was equal in HFD and ND mice. These later results suggest that obesity, unlike direct FFA-exposure, might not utilize the AMPK’s signal pathway. However, in the murine obesity model mTOR was markedly activated, potentially suppressing the effect of FFA (diet) on autophagy.  

Figure 13: Regulatory signals involved in the mechanism underlying proteinuria-induced autophagy in PTC’s. (B,C) LC3-II expression of PTC transfected with siRNA directed against the TSC1, AMPKa, and Sirt1 genes after serial IP injections of palmitate-bound albumin; (D) PS6K expression in response to palmitate-bound albumin injection; (F) AMPK activity in response to palmitate-bound albumin injection.  

Finally, to test the effect of autophagy on in vivo cell viability, Yamahara et. al., used renal Atg5 knock out mice to assess PTC damage. Atg5 is a necessary component in the induction of autophagy and in these experiments, Atg-5 knockout mice was used to provide a model of FFA-albumin and obesity induced toxicity in the absence of
autophagy. FFA-albumin-induced histological damage was greater in Atg5 knockout mice compared to control. In addition, apoptosis was significantly increased in Atg5 knockout mice as evidenced by Western blot analysis of caspase-3 and poly ADP ribose polymerase (PARP) cleavage: common apoptotic markers.47

Figure: 14: Atg5 knockout mice showed greater histological damage in vivo and increased apoptosis in FFA-albumin overloaded PTC’s in vitro. (E) Histological damage score of Atg5+/+ and Atg 5-/− mice overloaded with FFA-rich albumin or PBS; (G-I) Western blot of cleaved PARP and caspase 3 expression in Atg5+/+ and Atg 5-/− mice exposed to FFA-rich albumin or PBS.47

Yamahara et. al., showed that the excess reabsorption of FFA bound to albumin plays an important pathogenic role in the PTC injury in proteinuric kidney disease. Furthermore, the most likely mechanism responsible for FFA-induced autophagy appears to involve AMPK. Yamahara et. al., also suggested that obesity impairs the ability of FFA’s to induce autophagy and that hyperactivation of mTOR most likely mediates this impairment. Impaired autophagy in obese and Atg5 -/- mice exacerbated damage in FFA-overloaded proximal tubules (in vivo). This suggests that autophagy is protective against FFA-damage to the proximal tubule.47
DISCUSSION

The studies by Yamahara et al. and Wei Jing Lui et al. provide important initial steps for understanding the effect of autophagy in proximal tubule injury that accompanies proteinuric kidney disease. However, these provocative findings must be interpreted in the broader context of our current understanding of proximal tubule injury in CKD. The relationship between excess reabsorption of protein, the oxidative and ER stress resulting from increased endocytosis, as well as between autophagy and apoptosis is essential for appreciating the significance of autophagy in CKD. This discussion will attempt to compare and contrast these two emerging bodies on information.

THE ROLE OF PROTEIN REABSORPTION IN PROXIMAL TUBULE INJURY

Wei Jing Lui et al. showed that LC3-II and SQSMT1 expression increased in a dose-dependent manner after exposure to MCNS proteins. The adaptation of endocytic mechanisms in response to an increased protein load has been intensively studied however, much of the research is complex and inconclusive. It seems that the overall adaptive response to proteinuria initially includes increased protein reabsorption in which protein is both endocytosed and degraded at a faster rate, resulting in a new steady state. Unfortunately, this mechanism is imperfect and lysosomal degradation cannot keep pace with the high capacity MCA mediated endocytosis. The result is the abnormal accumulation of endosomes containing toxic contents. When levels of proteinuria become too high (i.e., >4mg/ml in mice), the PTC is forced to downregulate megalin
expression in an attempt to inhibit further protein entry into the cell.\textsuperscript{68,75} The behavior of the cell suggests an important “struggle” between the organism’s need to reduce urinary protein loss the PTC’s need to avoid the toxic effect of protein reabsorption.\textsuperscript{68}

This switch to down regulation of endocytosis could be an important precursor to cell death.\textsuperscript{68} Wei Jing Lui et. al., showed that peak LC3-II and LAMP 2 expression was seen at 8 hours before declining for the remainder of a 24 hour observation period. In the first 8 hours of protein exposure, proximal tubule endocytosis increased to account for the increased protein load.\textsuperscript{27} During this time period, autophagy was induced in response to cellular stress, presumably to minimize the untoward effects of the excess protein and dysfunctional cytoplasmic elements.\textsuperscript{28} In addition to activation of autophagy, the increase in cell stress also induced apoptosis.\textsuperscript{28} Coincidently, induction of apoptosis has been shown as early as 6 hours after protein exposure and increased exponentially over a 24 hour period in immortalized HK-9 cells exposed to 10mg/ml of FFA-rich albumin.\textsuperscript{51}

\begin{figure}[h]
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\includegraphics[width=\textwidth]{figure15.png}
\caption{Apoptosis is seen as early as 6 hours after administration of FFA-rich albumin (10 mg/ml) to HKC-9 cells.\textsuperscript{51}}
\end{figure}

Therefore, initial exposure to high concentrations of proteins causes activation of autophagy. This pro-autophagic response peaked 8 hours at which point the cells commit to apoptosis.\textsuperscript{27} In summary, the decrease in LC3-II expression after 8 hours exposure to
8mg/ml FFA-rich albumin could be the result of a decrease in autophagy rather than to activation of apoptosis.

In addition to excess protein load the renin-aldosterone-angiotensin system (RAAS) also regulates endocytosis in the proximal tubule cell. Recently, angiotensin-II has been shown to act through Akt to increase endocytosis in the proximal tubule.\textsuperscript{69} The effect of ANGII on endocytosis is quite complex due to the fact that the two ANGII receptors, AT\textsubscript{1} and AT\textsubscript{2}, sometimes exhibit competing roles.\textsuperscript{70,71} AT\textsubscript{1} receptors are the prominent angiotensin receptor type in adult PTC cells. AT\textsubscript{1} receptors seem to down-regulate megalin expression and inhibit endocytosis.\textsuperscript{73} In contrast, AT\textsubscript{2} receptors activate PKB and increase endocytosis. Although conclusions are in conflict, it seems that ANGII administration increases proximal tubule endocytosis.\textsuperscript{74} Thus, despite being the less expressed receptor type, AT\textsubscript{2} activation of Akt seems to be the predominant signal involved in ANGII regulation of endocytosis in this structure.

HK-2 exposure to MCNS proteins was sufficient to induce autophagy and that activation of autophagy was not seen with FFA-depleted HSA exposure.\textsuperscript{27} During increased reabsorbtion of protein, the PTC increases autophagolysosome proteolytic enzymes, such as cathepsin B and L. Unfortunately there seems to be no increase in the activity of lysosomal acid lipases responsible for degradation of cholesterol esters and triglycerides.\textsuperscript{50} Therefore, in the absence of an increase in activation of acid lipases than, increased albumin degradation results in inevitable, free fatty acids accumulation in the PTC.\textsuperscript{39,50,52} Yamahara et. al., showed that accumulation of FFA-rich albumin not albumin itself induced autophagy in PTEC.\textsuperscript{47} It has been demonstrated that there is no
significant difference in reabsorption between FFA-rich albumin and FFA-depleted albumin.\textsuperscript{78} This seems to indicates that there is no feedback mechanism that directly down-regulates reabsorption in response to excess FFA accumulation in the proximal tubule.

Obesity plays an important role in the ability of mice PTC to induce autophagy in response protein excess through hyper-activation of mTOR. Although FFA’s bound to albumin may be the primary factor that activates autophagy, findings in these obese mice suggests that there are other factors that can attenuate FFA’s activation of autophagy. Yamahara et al., suggested that hyper-insulinemia in the HDF mice might inhibit autophagy. Although insulin mediated mTOR activation has been shown in podocytes, it has yet to be studied in PTC.\textsuperscript{56} Another protein that could explain the inhibitory effect of obesity on autophagy is epidermal growth factor (EGF). EGF is one of the hormones and growth factors that are excessively excreted by adipocyte in obese subjects.\textsuperscript{57} Increased filtration of EGF and increase in activation of the EGF-receptors are seen in the PTC in proteinuric kidney disease.\textsuperscript{58} Interestingly, both insulin growth factor and EGF- receptors are upstream regulators of MEK and ERK and have shown to inhibit autophagy by activating mTOR.\textsuperscript{76} The effect of insulin and EGF on autophagy in the PTC could provide a better understanding of diabetes induced CKD.
Figure 16: mTORC1 has upstream regulatory connections to PKB and other growth factors.\(^7\)\(^6\)

Another factor that could attenuate FFA induced autophagy is megalin. Interestingly, the cytoplasmic motifs of megalin binds to various “phosphorylation, signaling, and protein interaction motifs,”\(^15\) and might be able to regulate protein induced autophagy.\(^15\) One important signaling interaction in the MCA complex’s relation to autophagy is megalin’s recruitment of Akt to the intracellular side of the apical plasma membrane.\(^6\)\(^8\) Increasing the pool of active Akt on the cellular membrane and may help delay apoptosis and atrophy of the proximal tubule cells during proteinuria.\(^6\)\(^8\) Akt is also an up stream regulator of mTOR and can inhibit autophagy. Once activated, Akt phosphorylates TSC2. TSC2 forms a complex with TSC1 that binds and inhibits the mTOR complex. When TSC 2 is phosphorylated, it dissociated from TSC 1 removing the inhibition of mTOR and inhibiting autophagy.\(^2\)\(^8\) Megalin’s cytoplasmic domain also can regulate protein-protein interactions, most notably JNK interacting proteins 1 and 2 which are responsible for proper assembly of the c-Jun N-terminal kinase scaffold.
assembly.⁵⁸ Although megalin’s affect on c-Jun kinase is yet to be understood, this kinase is involved in bcl-2 phosphorylation and subsequent dissociation of bcl-2 from beclin-1, resulting in increased autophagy.²⁸ Neither the Wei Jing Liu et. al., or Yamahara et. al., investigated insulin action on IGF, EGF on EGR-R nor did they examine megalin-mediated activation of Akt to determine their role in autophagy. Since Wei Jing Liu’s study showed that generation of ROS accompanied autophagy, it seems doubtful horomonal signaling or megalin actions on akt are responsible for inducing autophagy. It seems more plausible that these signals play a role in attenuating autophagy. Yamahara et. al., suggested that regulation of mTOR could attenuate FFA-induced autophagy. Akt, megalin, IGF and EGF’s regulation of mTOR in CKD is a potential therapeutic target for upregulating autophagy in the PTC in order to attenuate protein-induced autophagy.
THE SOURCE OF ROS GENERATION IN PROTEIN OVERLOADED PTC’S RESPONSIBLE FOR INDUCING AUTOPHAGY

Wei Jing Lui et al., demonstrated that MCNS proteins generated reactive oxygen species. By reducing the levels of ROS by administration of antioxidants catalase and tiron, protein induced autophagy was abolished in HK-2 cells. Understanding oxidative stress in the proximal tubule is essential for interpreting the results of Wei Jing Lui et al and Yamahara et al’s studies. Oxidative stress plays a central role in the induction of other cellular responses to proteinuric kidney disease including tubulointerstitial injury, the intra-renal angiotensin system (RAS) and apoptosis in the PTC. Given the central role oxidative in tissue injury, identifying and modifying the source that causes ROS generation in proteinuric kidney disease is paramount for understanding and preventing damage in the proximal tubule.

Although there are many sources that can generate ROS species inside the cell, the most common sites are the PKC/NADPH oxidase complex, located on the plasma membrane, and the electron transport chain in the mitochondria. Furthermore, both NADPH oxidase and the mitochondria can change their production of ROS is response to pathologic factors present in the filtrate of subjects with CKD.

Initial research into oxidative stress in protein-induced cell injury in the PTC pointed toward activation of protein kinase C (PKC) and NADPH oxidase as the source of ROS generation. In search of potential proteins that induced oxidative stress, FFA-depleted human serum albumin and IgG were investigated and increased PKC activity in vitro. Activation of PKC in turn activates NADPH oxidase, and enzyme capable of generating large amounts of cytosolic ROS. Although the mechanism by
which these proteins activate PKC is not fully known, megalin has intracellular motifs that bind PKC. This could explain how two fundamentally different proteins like albumin and IgG, that both bind megalin, produce nearly identical amounts of ROS in protein-overloaded PTC.

Angiotensin II can also activate PKC. It is theorized that the interactions between megalin, Akt, PKC, and ANGII create a “vicious cycle” that promote cell autophagy and apoptosis. With lower concentrations of excess albumin in the filtrate, megalin reabsorbs excess protein leading to recruitment and activation of Akt, thereby promoting cell survival. Megalin can also activate PKC and NADPH oxidase to generate ROS. These ROS species increase PTC expression of angiotensin converting enzyme, angiotensinogen and angiotensin receptor 1. In fact, the proximal tubule is capable of producing ten-fold greater local ANGII concentrations than seen in plasma. ANGII can further activate Akt and PKC leading to up-regulation of endocytosis and further expression of ANGII, respectively. The results in a cycle that promotes excess protein endocytosis, as well as increased ANGII and ROS production. This loop is interrupted at high intracellular concentrations of protein after megalin expression is inhibited and the interaction between megalin and Akt is reduced. The loss of Akt recruitment, and a fall in Akt enzyme activity, coupled with activation of TSC1/2 complex, inhibits mTOR, ultimately inducing autophagy. Furthermore, the generation of ROS via PKC can also activate autophagy through AMPK, p53, JNK1 PERK, and Atg4.
Figure 17: Regulatory interactions of PKB, PKC, and the RAS may cause a “vicious cycle” of ROS production and excess albumin endocytosis.

This feedback loop highlights an important balance between the organism’s need to reduce the loss of protein in the urine by up-regulating endocytosis while avoiding the toxic effect of excess protein; the later involves down-regulation of megalin and PKB at high concentrations of protein.  

PKC and NADPH oxidase were promising targets as the major source of oxidative stress in the proximal tubule cell. But recent evidence has shown a shift toward the mitochondrion as the main source of ROS generation in protein-induced proximal tubule damage. In 2007, Erkan et al. demonstrated that inhibition of PKC’s α subunit reduced HSA induced apoptosis. However; inhibition of PKC’s δ did not have any effect on HSA induced PTC apoptosis. Thus, PKC’s effect on apoptosis is not through generation of ROS but, due to increasing reabsorption of HSA, most likely through activation of the RAS system. Mounting evidence for FFA bound to albumin as the
dominate cause of PTC damage in CKD further cast doubt on PKC activity as the leading cause of ROS generation in proteinuric kidney disease. A study by Ishola et al. in 2006, showed that albumin bound with oleic acid generated pathological quantities of ROS. Inhibitors of NADPH oxidase had no significant effect on oleic acids ability to generate ROS; indicating that PKC was not the site of FFA induced ROS generation. Furthermore, mitochondrial respiratory chain inhibitors severely reduced oleic acid generated ROS.$^{78}$ A recent study by Muhammad U. Cheema et al. showed that in vivo administration of ANGII alone was not sufficient to induce increased expression of LC3-II in PTCs.$^{74}$

In spite of the fact that megalin and ANGII activate PKC/NADPH oxidase, this seems to be an unlikely source of the ROS seen in Wei Jing Lui et al’s study. ANGII receptor inhibitors have a profound effect on reducing oxidative stress in the PTC and slowing the progression of CKD.$^{67}$ There are currently no studies investigating ANGII effect on autophagy in the PTC in a proteinuric model, however based on current research, the most likely mechanism in which ANGII can effect albumin induced autophagy is through regulation of reabsorption and not through generation of ROS. Therefore, it is most likely that ANGII receptor blockers reduce ROS indirectly by decreasing the reabsorption of FFA-bound albumin.

Yamahara et al showed that the FFA’s bound to albumin induced autophagy in the PTC in vivo.$^{47}$ This follows other research that suggests that excess FFA uptake is the dominant pathological factor leading to damage to the proximal tubule.$^{60,63,77-79}$ Therefore the most likely factor in the MCNS proteins that resulted in the oxidative stress
needed to activate autophagy in the Wei Jing Luis study was the free fatty acids bound to the excreted albumin.

After cleavage from albumin, FFA’s are metabolized into triglycerides with other minor sources being phosphatidylcholines, diacylglycerols and phosphotidylenothotols.\textsuperscript{79} Even when exposed to nephrotic levels of FFA-bound albumin, un-metabolized FFA in the cytosol, represent a small fraction of the overall FFA influx into the cell. Furthermore, most of these cytosolic FFA are bound to liver-type fatty acid binding protein (L-FABP).\textsuperscript{79,80} Interestingly, L-FABP, largely responsible for delivering FFA to the mitochondria, is excreted from PTC’s in proteinuric kidney disease. This demonstrates and important mechanism of how the PTC responds to FFA excess by excreting FFA bound to L-FABP into the filtrate. Furthermore, L-FABP can be measured in the urine as a clinical marker of proximal tubule stress in patients with CKD.\textsuperscript{80}

FFA have many diverse mechanisms for inducing oxidative stress in the proximal tubule, however recent evidence points to excess $\beta$-oxidation in the mitochondria as the most likely sources of ROS generation.\textsuperscript{81} Only 4.6% of the total reabsorbed C\textsuperscript{14} labeled palmitate-albumin undergoes beta-oxidation and conversion to CO\textsubscript{2} in cultured opossum kidney cells. Palmitate and oleic acid show the greatest propensity for $\beta$-oxidation in the proximal tubule with palmitate undergoing more rapid oxidation compared to oleic acid.\textsuperscript{79} However; by analyzing early diabetic mice PTCs a recent study demonstrated the most likely source of ROS generation is an undiscovered site on the electron transfer flavoprotein (ETF) and ETF-coenzyme Q oxidoreductase (ETF/ETF coQ).\textsuperscript{81} (ETF/ETF
coQ) undergoes rapid changes in redox state and is responsible for transference of electrons from the FADH₂ produced by the first dehydrogenase reaction of β-oxidation. This rapid change in redox state produces superoxide, which is transformed into H₂O₂ by superoxide dismutase.

Under normal production of ROS in the mitochondria, cell antioxidants such as catalase are deployed to eliminate these species. The excess ROS generation from FFA oxidation overwhelms the antioxidant system in the mitochondria and causes a vast change in the redox environment of the cell. In addition, FFA-depleted albumin causes a compensatory increase in mitochondrial antioxidant proteins in HK-2 cells, but this compensatory reaction was not seen in HK-2 cells exposed to oleic acid bound albumin. Furthermore, FFA in the cytosol can react with mitochondrial generated ROS to create FFA radicals.

To summarize, in spite of being a small fraction of the overall fate of FFA absorption into the PTC, β-oxidation is the source of extensive ROS production in in vitro models of proteinuric kidney disease. The proximal is unequipped to handle the increased load of FFA and resulting oxidative stress. The PTC fails to respond to increase FFA endocytosis because its inability to significantly increase the expression of acid lipase when FFA-albumin is first reabsorbed. Furthermore, there seems to be no feedback mechanism that responds to FFA excess to regulate the PTC’s reabsorptive mechanism. The PTC does however attempt to account for FFA overload by excreting L-FABP to decrease the cytosolic FFA concentration. Although the FFAs bound to albumin are mostly metabolized into other forms such as triglycerides, the FFAs
that do remain are enough to cause significant ROS generation when oxidized in the mitochondria.\textsuperscript{79,80,81} The subsequent oxidative stress from FFA oxidation is a necessary step to induce autophagy in the PTC.\textsuperscript{27}

\textit{AMPK Activation Due to the Effects Oxidative Stress Is Responsible For Activation of Autophagy in FFA-Overloaded PTC’s}

Wei Jing Liu et al. determined activation of autophagy is a consequence of oxidative stress induced by protein excess in the PTC.\textsuperscript{27} The ROS and free radicals produced from FFA oxidation leads to expression of cytokines, DNA damage, and protein misfolding.\textsuperscript{28} Both Wei Jing Lui et al. and Yamahara et al. failed to clearly determine which one of these effects from oxidative stress activate autophagy.\textsuperscript{27,47} Whatever mechanism is responsible for ROS activation of autophagy, Yamahara et al. demonstrated that AMPK is likely involved.

ROS causes inappropriate oxidation of amino acid residues in newly forming proteins in the ER. These proteins misfold and aggregate in the endoplasmic reticulum. The ubiquitin proteasome system (UPS) is used to degrade misfolded and dysfunctional proteins inside the cell. Large amounts of misfolded proteins aggregate in the ER and put strain on the UPS. ER stress can be a potent stimulator of autophagy.\textsuperscript{28,54} Three ER membrane bound proteins; PERK, ATF6, and IRE1; have shown to regulate many proteins responsible for stimulating autophagy; although none of these proteins appear to be AMPK.\textsuperscript{28,54} In addition, excess protein misfolding also causes disruption of calcium homeostasis resulting in leakage of calcium out of the ER and into the cytosol. Calcium\textsuperscript{2+}/calmodulin-dependent kinase kinase-\(\beta\) activates AMPK in response in increases in cytosolic calcium.\textsuperscript{54} Although this is a promising mechanism, the results
from Wei Jing Lui et al. and Yamahara et al. indicate that ER stress is not responsible for FFA induced autophagy in proteinuric kidney disease.\textsuperscript{27,47} Yamahara et al. demonstrated that palmitate activated PERK in response to ER stress but inhibition of ER stress did not effect LC3-II expression in response to FFA-albumin overload.\textsuperscript{47} Interaction between ER stress and autophagy was further discredited in the Wei Jing Lui et al study. MCNS proteins did not effect activation of autophagy through proteasome inhibition with MG132.\textsuperscript{27}

In addition to protein misfolding, oxidative stress can cause free radical damage to DNA.\textsuperscript{28} PARP and ataxia-telangiectasia mutated (ATM) protein kinase are important repair enzymes that respond to oxidative damage to DNA.\textsuperscript{83,84} ATM and PARP also act through AMPK to induce autophagy.\textsuperscript{83,84} PARP probes DNA for single strand breaks and produces a multi ADP-ribose chain. This ADP-ribose chain recruits other DNA repair enzymes to fix the break.\textsuperscript{85} Creation of the ADP-ribose chains is a significant energy depleting process that rapidly depletes NAD\textsuperscript{+}, impairs ATP production, and causes of DNA damage resulting in cell death.\textsuperscript{83,85} The shift in the AMP/ATP ratio as a result of PARP activation is sensed by liver kinase B1 (LK-B1), an important upstream regulatory protein of AMPK. In addition to LKB1 activation of AMPK in response to shifts in the AMP/ATP ratio, AMP can allosterically activate AMPK.\textsuperscript{28,83,85} Atg5 knock out mice in the Yamahara et al. study demonstrated that degreee of cleaved PARP was greater in FFA-albumin injected mice compared to control.\textsuperscript{47} Densitometry analysis of this western blot showed no distinct change in PARP expression between any of the groups. Although this indicates that PARP expression may not be effected by FFA-induced DNA
damage, a PARP1 activity assay, measuring biotinylated NAD+ incorporation into PARP generated poly ADP ribose chain, would determine the activity of PARP in response to FFA-induced oxidative stress.\textsuperscript{47}

ATM is another important DNA damage repair enzyme that works with PARP to detect DNA strand breakage and activates DNA repair enzymes to repair the breaks. Unlike PARP, ATM can be subject to direct oxidation by free radicals and ROS.\textsuperscript{84} Oxidation of cytosolic ATM phosphorylates LK-B1 as well as activates AMPK directly.\textsuperscript{28,84} This provides a mechanism for direct activation of autophagy in response to oxidative stress. Therefore, autophagy can be activated directly by changes in oxidative stress and respond to subsequent DNA damage and ER stress cause by ROS production to be significant enough to activate autophagy.

Tubulointerstitial inflammation and fibrosis is a hallmark of proximal tubule damage in subjects with chronic kidney disease.\textsuperscript{4} In response to heavy protein exposure, the PTC release various cytokines and inflammatory markers; RANTES, MCP-1, fibronectin and IL-8 are among some of the cytokines released in response to protein overload by PTCs. The release of these cytokines is produced largely from activation of the transcription factor NF-κB.\textsuperscript{44,58,86,87,90} NF-κB is constitutively inhibited by inhibitor of kappa B (IκB) and activated when IκB is phosphorylated and deactivated by IκB kinase (IKK). IKK responds to a multitude of different stresses including DNA damage and oxidative stress. Constitutively activated IKK subunits can also activate autophagy through AMPK and JNK1.\textsuperscript{28} IKK activation provide another mechanism by which oxidative stress directly activates autophagy through AMPK.
Generation of ROS has been shown to increase the expression of TGF-β mRNA in mesangial cells.\textsuperscript{88} Although the ROS-dependent increase in TGF-β expression has never been tested in PTCs, the “localization of TGF-β mRNA and protein within tubular epithelial cells”\textsuperscript{89} has been demonstrated in patients with heavy proteinuria.\textsuperscript{55,89} Not only is TGF-β an important cytokine in the inflammation and fibrosis seen in proteinuric kidney disease, but can activate AMPK through one of its receptors, TGF-β activated kinase-1.\textsuperscript{28}

It has yet to be determined whether PARP, ATM, TGF-β, or IKK are the regulatory proteins linking the oxidative stress to AMPK. Further research into these regulatory proteins is key in discovering how FFA bound to albumin induces autophagy in the PTC.

\textit{CYTOPROTECTIVE ROLE OF AUTOPHAGY}

Although much of Yamahara et al and Wei Jing Lui study was to investigate whether protein overloaded PTC’s induced autophagy, both studies investigated the effect of autophagy in the survivability of the cell. The results from both studies indicate that activation of autophagy resulted in an increase in cell survivability and a decrease in proximal tubule damage.\textsuperscript{27,47} In Yamahara et al’s study Atg 5 konckout mice showed increase signs of proximal tubule damage and increased expression of caspase 3 cleavages.\textsuperscript{47} The Wei Jing Lui et al. study showed that protein activated the apoptotic factors HAVCR1 and LCN2. The activation of these factors increase when autophagy was inhibited by chlorquine and decreased when autophagy was upregulated by rapamycin.\textsuperscript{27}
Other research into autophagy in the proximal tubule provide insights into how autophagy is cytoprotective in protein overloaded PTC's. A recent study by Kimura et al. investigated PTC damage after administration of cyclosporine A, a powerful immunosupressant that also causes mitochondrial dysfunction and increased oxidative stress. Mitophagy, or autophagy of mitochondria, is a natural part of cell homeostasis responsible for elimination of dysfunctional mitochondria.\textsuperscript{28,91} An increase in dysfunctional mitochondria can cause oxidative stress and a decrease in proper energy production.\textsuperscript{91,92} Kimura et al showed that autophagy deficient mice PTCs had a decrease survival ratio when compared to wild type mice after administration of cyclosporine A (CSA).\textsuperscript{91}

To test if autophagy effected CSA toxicity in the mitochondria, Kimura et al. looked at the mitochondrial membrane potential and generation of mitochondrial ROS.\textsuperscript{91} A change in the mitochondrial membrane potential can lead to induction of apoptosis and inhibition of oxidative phosphorylation.\textsuperscript{93} The results of the Kimura et al study showed that CSA administration cause significantly more ROS generation in autophagic deficient mice than in control mice. Furthermore, the membrane potential in autophagic-deficient cells was less than the baseline wild type mice. In addition, CSA reduced membrane potential to a greater extent in autophagic deficient mice than in wild type mice.\textsuperscript{91}

The most likely explanation for these results is that the autophagic deficient mice where unable to remove any dysfunctional mitochondria that might cause damage to the cell. This leads to an increase in the total number of mitochondria
before administration of CSA. The large number of mitochondria in the autophagic deficient cell makes the cell more vulnerable to CSA administration. CSA treatment promotes apoptosis my changing the mitochondrial membrane potential and creating large amounts of ROS are produced which damage the cell. In conclusion, one of the reasons why autophagy might alleviate the FFA-induced autophagy in the PTC of subjects with CKD is by destroying dysfunctional mitochondria that generate the majority of the ROS responsible for proximal tubule damage.

Yamahara et al. and Wei Jing Lui both showed that FFA-albumin overloaded cells cause protein misfolding and ER stress. They also showed that the ER stress instigated by FFA-albumin was not responsible for inducing autophagy. Even though Autophagy may not be activated by ER stress, it does not mean that autophagy cannot help degrade the misfolded proteins and alleviate the ER stress in proteinuric kidney disease. In fact studies of liver cells showed that aggregated mutant fibrinogen in the ER was by in large part cleared by autophagya after the fibrinogen aggregates had overwhelmed the UPR system. A similar finding was observed in C2C5 cells with aggregates of dysferlin, an important protein mutated in human muscular dystrophy. Inhibition of autophagy in C2C5 Atg-5 knock out cells showed an increase in dysferlin aggregates in the ER. α1-antitrypsin Z is another protein that shows increase aggregation in liver cells in the absence of autophagy. It is not clearly clear what proteins are prone to aggregate in FFA-albumin overloaded PTCs or what effect FFA-albumin has on apoptosis. Both Yamahara et al and Wei Jing Lui et al. did not investigate the effect of autophagy on ER stress and
whether elimination of the misfolded proteins by autophagy has any effect on PTC survival. This could be a source of future research and help in the understanding of the cytoprotective effects of autophagy in the proximal tubule.

The effect of autophagy on the proximal tubule in chronic kidney disease is largely uncertain. Only the studies by Wei Jing Lui et al. and Yamahara et al. have investigated the cytoprotective effects of autophagy. The study of agents that modulate autophagy could prove to be incredible fruitful for future research. Not only would they result in an understanding of how autophagy exerts a cytoprotective role against FFA-albumin induced autophagy, but could an important therapeutic target for decreasing damage to the proximal tubule and help to slow the progression of CKD.
CONCLUSIONS

Chronic Kidney Disease is a substantial health problem providing a substantial burden to the US health care system. Proteinuria is an independent risk factor for cardiovascular disease and progression to end stage renal disease in patients with CKD. Excess filtered protein in the filtrate reabsorbed in the PTC is the dominant source of pathologic damage resulting in patients with proteinuria. The most significant protein causing the pathologic damage to the PTC is albumin, more specifically the FFA’s bound to albumin. The excess reabsorption of FFA in the PTC causes and increase in oxidative stress mainly from the generation of ROS from the mitochondria. This oxidative stress generated by the mitochondria in response to increase FFA oxidation causes protein misfolding leading to ER stress, DNA damage, and release of cytokines and proinflammatory factors. These factors contribute to induction of apoptosis and ultimately cell death of the PTC.

Autophagy is a naturally occurring catabolic processes that response to cellular stresses in order to promote survival. It does this by catabolizing cellular organelles and other cytosolic components to provide energy in times of starvation and to eliminate dysfunctional substances causing stress inside the cell. Two recent studies by Wei Jing Lui et al. and Yamahara et al. have investigated the role of autophagy in protein overloaded proximal tubule cells. Their findings make some important conclusions about the role autophagy in the PTC has on CKD.27,47

These studies as well as other current research on proximal tubule damage resulting from protein overload indicated that that FFA bound to albumin and the
resulting oxidative stress is the most likely protein cause of activation of autophagy in the PTC. However, it has yet to be determined whether activation of autophagy is in response ROS generation itself or from the effects of oxidative damage such as inflammation or DNA damage. What Yamahara et al. and Wei Jing Lui et al. both demonstrated was that activation of autophagy was not the result of ROS-induced protein misfolding causing ER stress. Yamahara et al. also demonstrated that whatever the underlying mechanism activation AMPK was most likely involved.27,47

Lastly, Yamahara et al. and Wei Jing Lui et al. demonstrated that activation of autophagy is protective against FFA induced toxicity in the proximal tubule. Novel therapy's enhancing autophagy in the PTC could provide novel therapies for treating patients with proteinuria and CKD.
REFERENCES


26. Yang, Z., & Klionsky, D. J. (2009). An Overview of the Molecular Mechanism of Autophagy. *Current Topics in Microbiology and Immunology*, 335, 1–32. doi:10.1007/978-3-642-00320-8_1


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