S5-1
Urinary exosomal molecules in acute kidney injury
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Urinary exosomes are secreted by tubular epithelial cells and these vesicles selectively contain functional molecules and/or disease-related molecules in the kidney. Therefore, urinary exosome research is expected to lead to the proposal of new physiological functions and the discovery of new diagnostic and therapeutic methods. Aquaporins (AQPs) are known to be expressed in a segment dependent manner and play a role in water homeostasis in the body. Of the known AQPs, AQP1 and AQP2 have been found in urinary exosomes (uAQP1, uAQP2). From 2006, we have examined uAQP release patterns in rat models of acute kidney injury (AKI), including ischemia/reperfusion (I/R), cisplatin (CIS) and gentamicin (GM) models. We here describe the remarkable results. The release of uAQP1 in the CIS and GM models was increased within 30 h after either CIS- or GM-treatment. In the GM model, the increase was possibly associated with the increased number of exosomes released into the urine. Release of uAQP2 in the CIS models was decreased 24 h after the treatment and this decrease was maintained for 7 days. In all animal models, both release of uAQP1 and uAQP2 were decreased in a phase of renal fibrosis, accompanied by their decreased renal expression. Recently, we also examined whether the miRNAs in exosomes (exo-miRs) can report the disease progression of I/R injury. The release of urinary exosomal miR-16, miR-24, and miR-200c in the injury state and miR-9a, miR-141, miR-200a, miR-200c, miR-429 in the recovery state were increased. Furthermore, we determined if exo-miRs in urine contain the sequence information that controls their sorting into exosomes. As a results, we found the candidate sequence of GGRS. We anticipate that these findings will contribute to the future clinical application of urinary exosomal molecules.

S5-2
Glomerular filtrate proteins induce the AKI-to-CKD transition in a mouse model of acute cardiorenal syndrome
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Acute cardiorenal syndrome (CRS-1) is a common complication of acute cardiovascular disease. Studies of acute kidney injury (AKI) to chronic kidney disease (CKD) transition, including patients suffering acute cardiovascular disease, report high rates of CKD development. Therefore, CRS-1 may associate with CKD. Heart-to-kidney signals transmitted by cardiorenal connectors have been postulated, but investigation into CRS-1 has been limited by technical limitations and a paucity of animal models. We used a translational model of CRS-1, cardiac arrest and cardiopulmonary resuscitation (CA/CPR), and reported findings from nanoscale mass spectrometry proteomic exploration of glomerular filtrate. Filtrate acquisition was confirmed by imaging, molecular weight and charge distribution, and exclusion of protein specific to surrounding cells. Filtration of proteins specific to the heart was detected following CA/CPR and confirmed with mass spectrometry performed using urine collections from mice with deficient tubular endocytosis. Cardiac LIM protein was a CA/CPR-specific filtrate component. Cardiac arrest induced plasma release of cardiac LIM protein in mice and critically ill human cardiac arrest survivors, and administration of recombinant cardiac LIM protein to mice altered renal function. These findings demonstrate that glomerular filtrate is accessible to nanoscale proteomics and elucidate the population of proteins filtered. The identification of cardiac-specific proteins in renal filtrate suggests a novel signaling mechanism in CRS-1. We expect these findings to advance understanding of CRS-1.

S5-3
Tubular inflammation via cGAS-STING pathway is a therapeutic target for Acute Kidney Injury
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Recognition of pathogen-associated molecular patterns or damage-associated molecular patterns via pattern-recognition receptors is critical for a host's defense, triggering signaling cascades that lead to innate immune response. Cyclic GMP–AMP synthase (cGAS)–stimulator of interferon genes (STING) pathway detects cytosolic DNA and induces innate immunity. We have recently reported that tubular mitochondrial damage leads to mitochondrial DNA leakage into the cytosol, via BCL-2-like protein 4 (BAX) pores on the mitochondrial outer membrane, activating cGAS-STING signaling and subsequent tubular inflammation in cisplatin–induced Acute Kidney Injury (AKI). Suppression of the STING ameliorates tubular inflammation and progression of AKI. Taken together, we conclude that therapeutic strategies targeting this pathway could be beneficial in preventing or treating AKI. In this session, I would like to talk about general information on the cGAS–STING pathway, our data, and perspective.
S5-4
Tertiary lymphoid tissue as a novel kidney injury marker and potential therapeutic target
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AKI to CKD progression has now been recognized as one of the most pressing unmet needs in renal medicine, highlighting the need for novel therapeutic strategies. Although more than one hundred therapeutic interventions have been successfully tested in animal models, no drug has been demonstrated to be effective for preventing progression of AKI to CKD in human patients. This lack of clinical translation has called into question the animal models being used. Because aging is one of the relevant risk factors for CKD progression after AKI, we previously induced AKI models to young and aged mice and found that aged but not young kidney exhibited tertiary lymphoid tissues (TLTs) after kidney injury. TLT is an inducible ectopic lymphoid tissue, which function as a site for lymphocyte activation. TLTs were also detected in human aged kidneys, with a composition similar to those of mouse TLTs. We have recently explored the potential of TLT as a novel histological marker reflecting kidney injury as well as a novel therapeutic target for various TLT-associated diseases. We have established a phenotypic evaluation method for TLTs in human kidneys based on the presumed developmental stages, and showed that the TLT stages were associated with the severity of kidney injury in mice and humans and could be reversible even after TLTs mature into advanced stages. We have also identified a key signaling pathway for TLT formation, which can be a novel therapeutic target for kidney diseases. In this session, we would like to discuss about TLTs in the kidney from two clinical point of views described above.

S5-5
Role of the DNA damage response in kidney tubular epithelial cells on acute kidney injury and subsequent CKD transition
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The regenerative potential of renal proximal epithelial tubular cells (RPTECs) is not unlimited and is dependent on the severity of injury. G2-M arrest of RPTECs was identified by our previous and others works, which promoted fibrosis and AKI-to-CKD transition through acquiring senescence-like phenotypes. This phenotype was bypassed by p53 inhibitors and DNA damage response (DDR) is thought to be an important factor of the AKI-to-CKD transition. However, there have been no studies in which modification of a DDR component in the kidney has been evaluated for its effect on the long-term response of the kidney to injury. Using a transcriptome analysis, cyclin G1 (CG1) was identified as an important mediator of G2/M arrest. We have identified formation of the target of rapamycin (TOR)–autophagy spatial coupling compartment (TASCC) in G2-M arrested PTECs both in vitro and in vivo. TASCC is a new compartment of the senescent cell and is formed from the fusion of late autophagosomes with lysosomes that contain mTORC1. Deletion of CG1 reduced G2/M phase cells, TASCC formation and fibrosis after injury in vivo. Prevention of TASCC formation in cultured PTECs blocked secretion of profibrotic factors. PTECs specific Raptor knockout reduced kidney fibrosis in mice. By using the mice with RPTEC deletion of the ATM– and Rad3-related (ATR), a master regulator of DDR we revealed that disruption of DDR resulted in more cumulative DNA damage, apoptosis, acute impairment of kidney function, and worse kidney fibrosis following injury. In addition, greater numbers of TASCC/cell were also found. These results were corroborated by in vitro studies of RPTECs and kidney organoids.

Our data indicate the DDR modulates the AKI-to-CKD transition through a pathway involving CG1 mediated G2/M arrest, TASCC formation and profibrotic factor secretion, as well as identifying novel therapeutic targets.