2014 JSSX Award for Young Scientists (Oct 22, 2014)

Kumamoto University

Molecular pharmacokinetic mechanisms of oxidative stress-induced tissue damage in chronic kidney disease for medical development and therapeutic application

慢性腎臓病における酸化ストレス臓器障害の分子動態学的解明と医療への展開

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Chronic kidney disease: CKD

- The prevalence of CKD in Japan is over 13 million patients, which is 13% among the Japanese general adults population.
- The number of patients with hemodialysis is exceeded 300,000.
- Dialysis costs more than 150 billion yen/year.
- CKD is a major risk factor for cardiovascular disease (CVD), which is a major cause of death in CKD patients.

Developing new therapeutic approaches for CKD is urgently necessary.

Outline

Therapeutic approaches to progression of CKD and CVD

1. Molecular mechanism of uremic toxin-induced tubular and vascular damage

- *p*-Cresyl sulfate induced-renal tubular and vascular cell damages
- CMPF induced-cell damage *via* generation of a radical intermediate

CKD/Uremic toxicity and CVD

2. Biomarker identification

- Monitoring of cysteinylated albumin by using ESI-TOFMS

3. Therapeutic development

- Novel anti-oxidative/anti-inflammatory agent: Albumin-thioredoxin fusion protein

Uremic toxins

Accumulated substances that interact negatively with biologic functions in CKD condition.

88 molecules were identified.

1.Low molecular weight molecules: 40 molecules

Guanidino succinate, Phenylacetic acid, Uric acid, Neopterin etc.

2.Middle molecules: 23 molecules

FGF23, PTH, β_2-MG, TNF-α etc.

3.Protein-bound solutes: 25 molecules

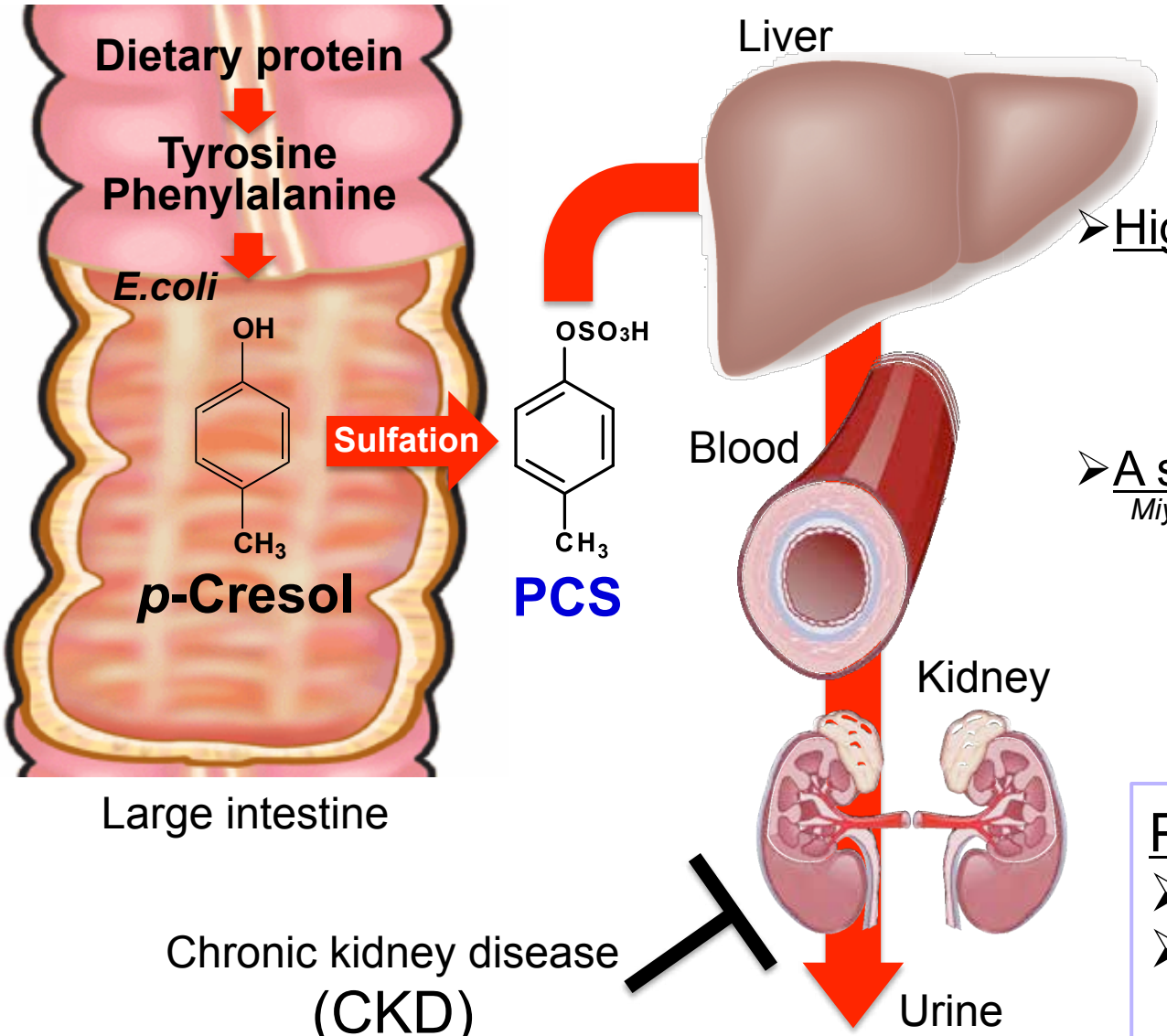
p-Cresyl sulfate, CMPF, Indoxyl sulfate, etc.

Insufficient removing by dialysis

Vanholder R et al. Kidney Int (2003)
Duranton F et al. JASN (2012)

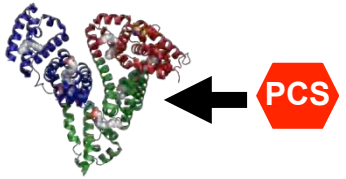
p-Cresyl sulfate: PCS

Martinez AW et al. CJASN 2005
De Loor H et al. Clin Chem 2005



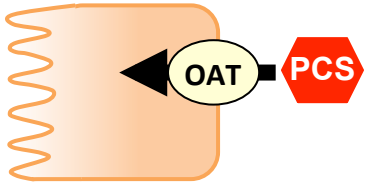
➢Highly bound to albumin

Watanabe H et al. DMD 2012



➢A substrate for OATs

Miyamoto Y, Watanabe H et al. NDT 2011
Watanabe H. Clin Exp Nephrol 2013



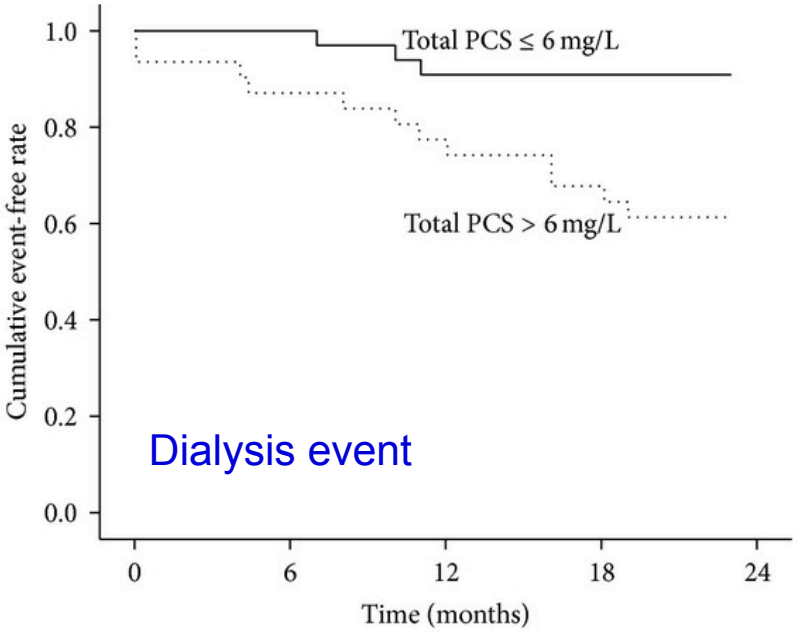
Plasma PCS level:

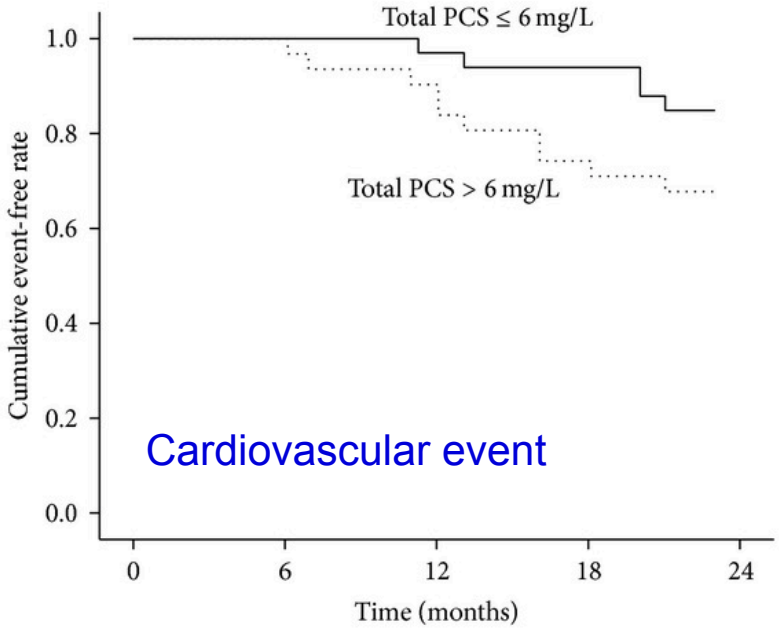
➢Healthy: <5uM

➢CKD stage5 HD: 200~300uM

Duranton F et al. JASN 2012

Plasma PCS levels associated with CKD progression, cardiovascular mortality





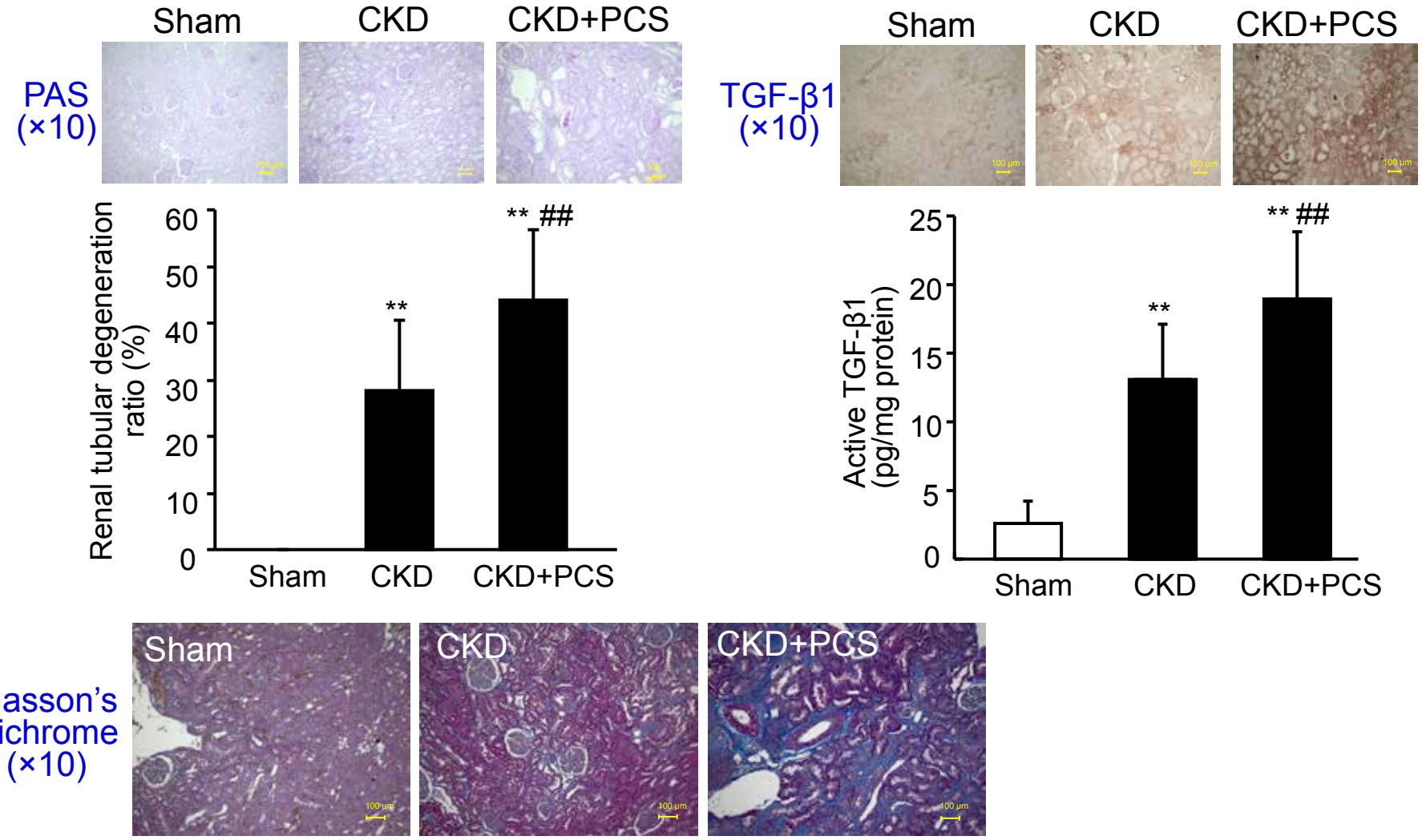
Clinical studies showed higher plasma PCS levels associated with 1.dialysis event and 2.cardiovascular event.

However, the biological functions of PCS in kidney/vascular system, and the mechanism of its action were largely unknown.

?

Lin CJ et al. Biomed Res Int. (2014)
Cheng JL et sl. Atherosclerosis (2012)
Wu I et al. NDT (2011&2012)
Liabeuf S et al. NDT (2010)
Meijers BK et al. CJASN (2010)
Bammens B et al. Kidney Int. (2006)

Histological evaluation of kidney from PCS-overloaded CKD rats

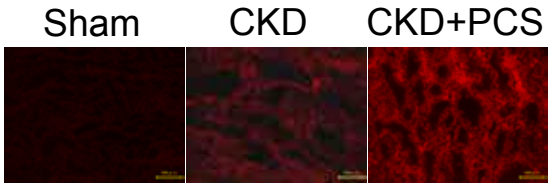


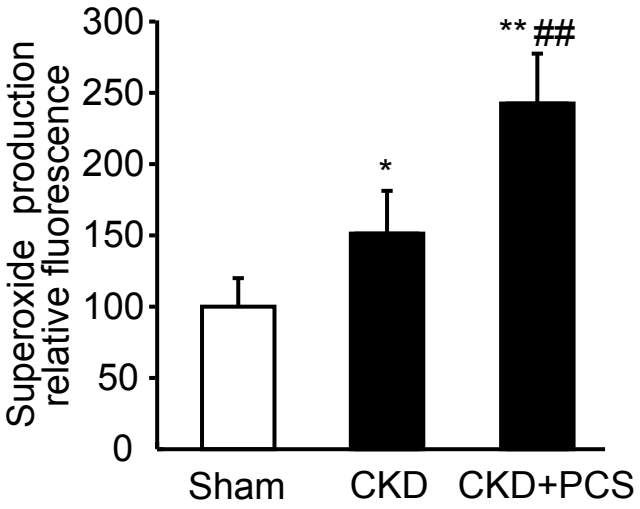
PCS-overload increased tubular damage, especially, tubular degeneration, TGF-β1 expression and renal fibrosis.

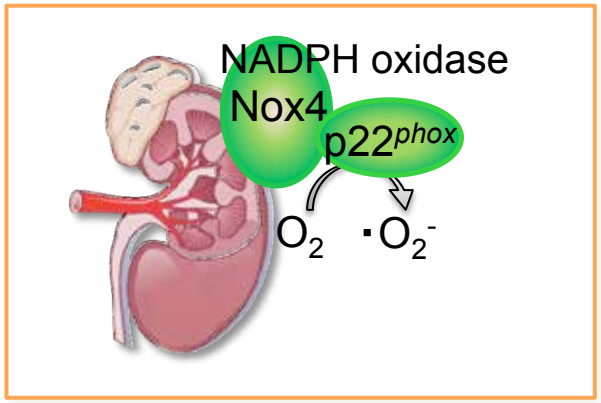
Watanabe H, et al. *Kidney Int.* 2013

Superoxide production and NADPH oxidase activity in kidney from PCS-overloaded CKD rats

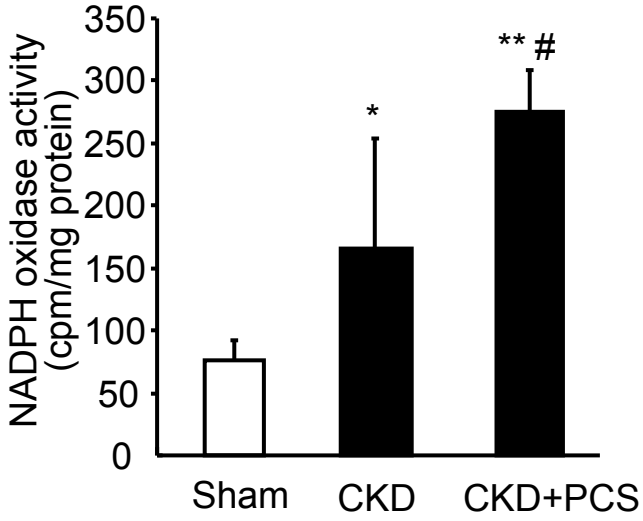
Superoxide production (DHE stain)







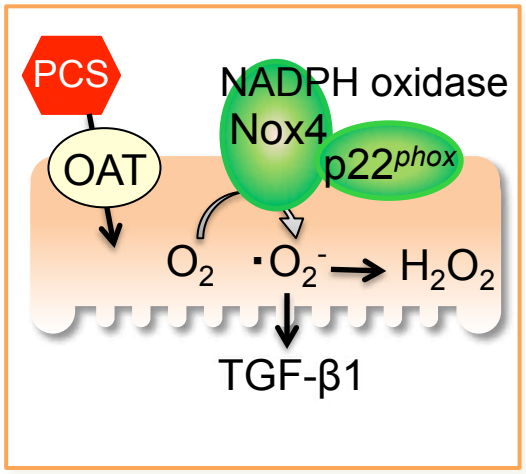
NADPH oxidase activity



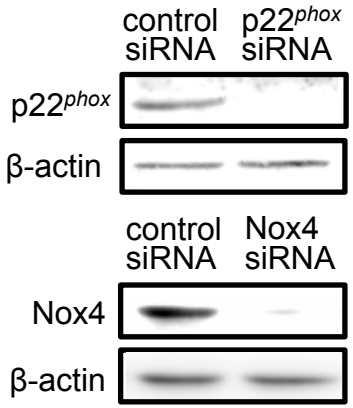
PCS-overload showed significant increase in superoxide production and NADPH oxidase activity.

Watanabe H, et al. *Kidney Int.* 2013

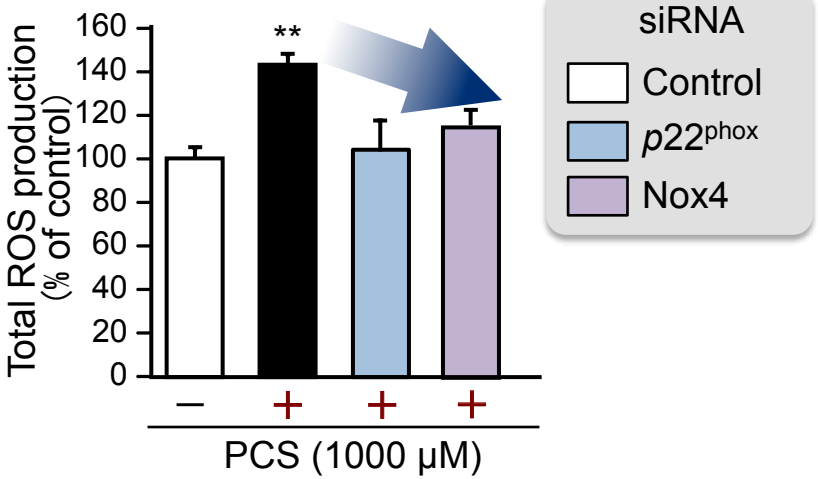
siRNA for NADPH oxidase and probenecid suppressed PCS-induced ROS production and TGF-β1 expression (HK-2)



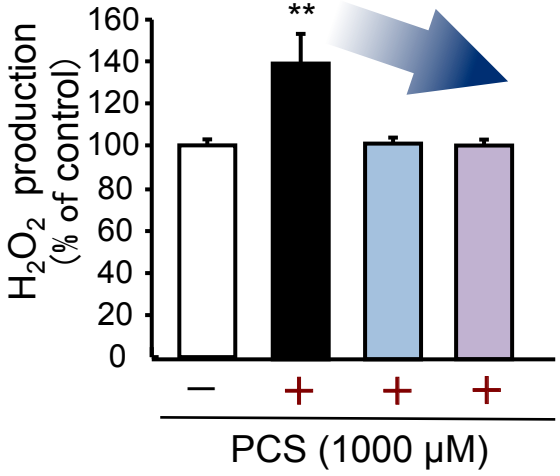
Nox4/p22^{phox} knockdown



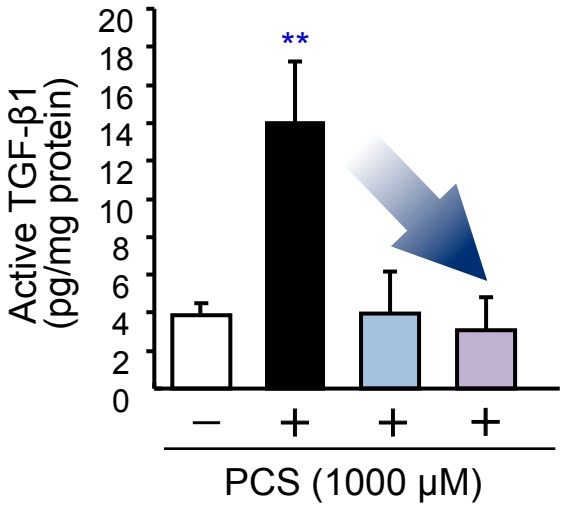
Total ROS production



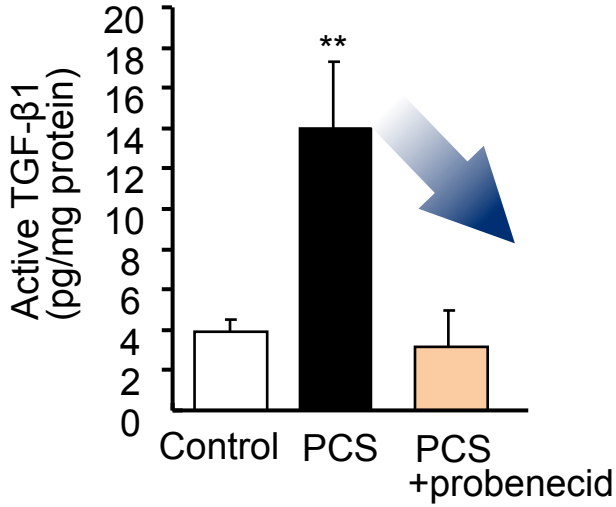
H_2O_2 production



Active TGF-β1 protein



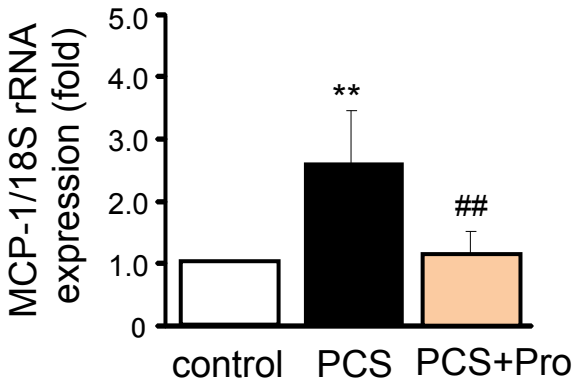
Active TGF-β1 protein

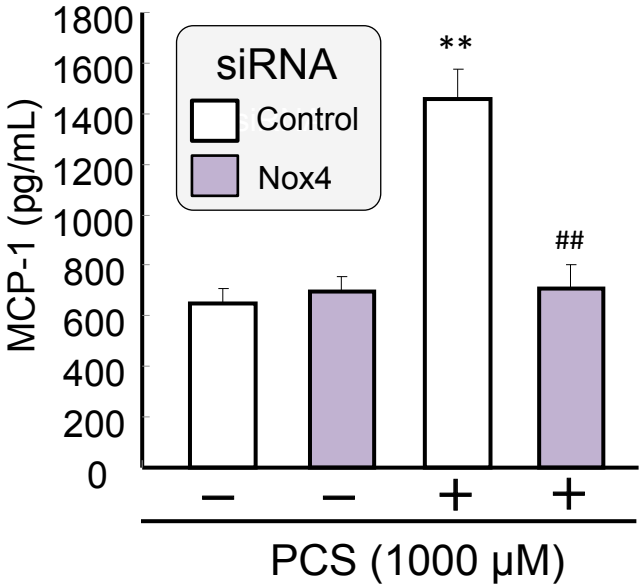


Cellular accumulation of PCS *via* OATs activates NADPH oxidase, which contributes to ROS production and increasing TGF-β1 expression.

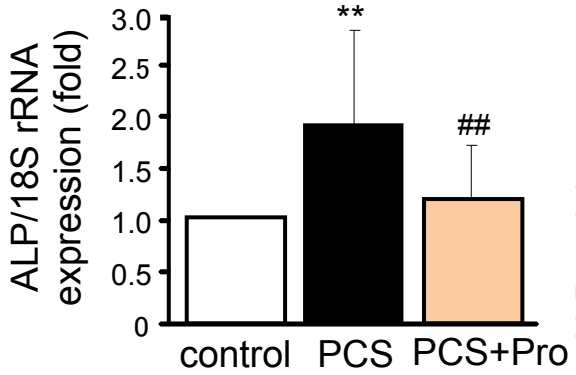
PCS increased MCP-1 expression in HUVEC and osteoblast-specific genes in HASMC

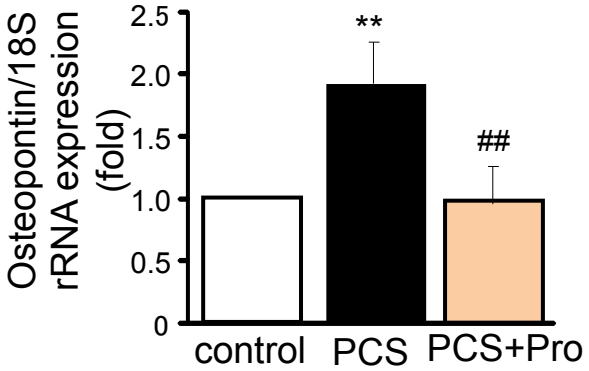
Vascular endothelial cell (HUVEC)

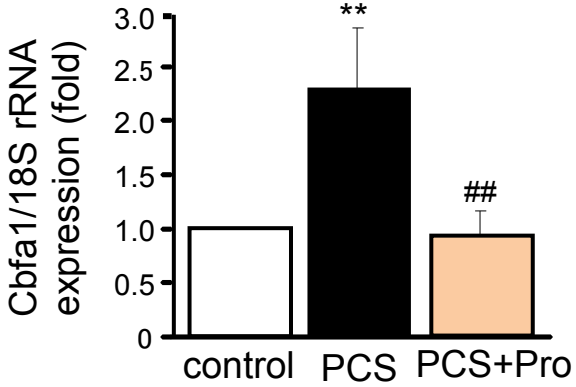


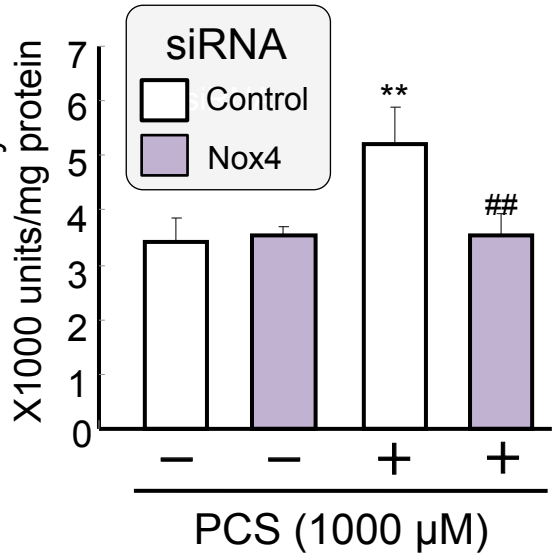


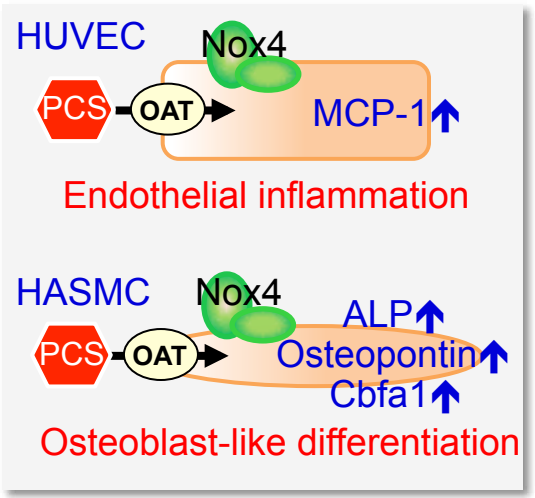
Vascular smooth muscle cell (HASMC)





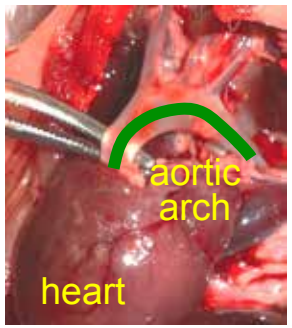


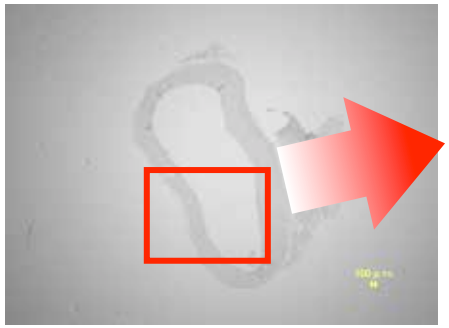




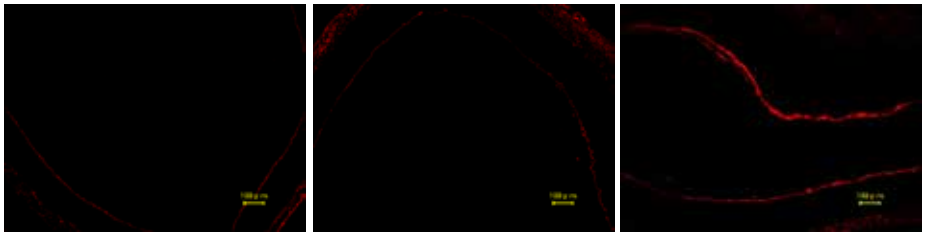
Watanabe H et al. *Pharmacol Res Perspect.* 2014

PCS-overload leads to vascular damage in CKD rats

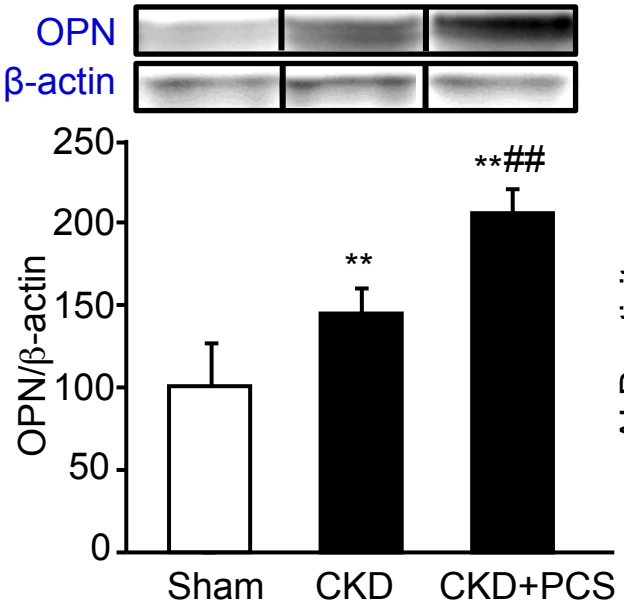




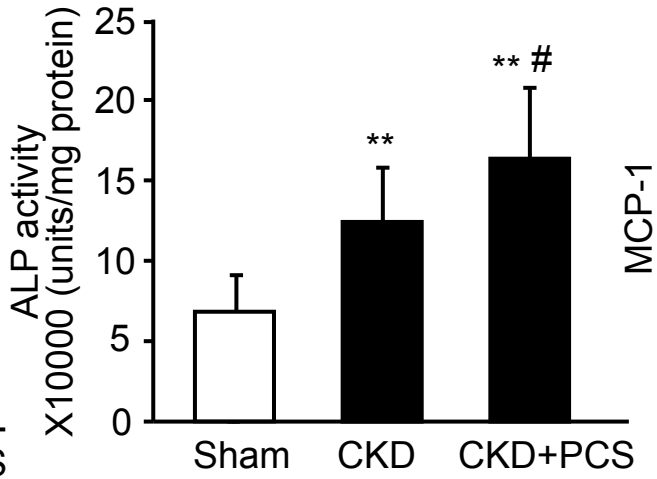
NADPH oxidase (Nox4) (x10) (aortic arch)



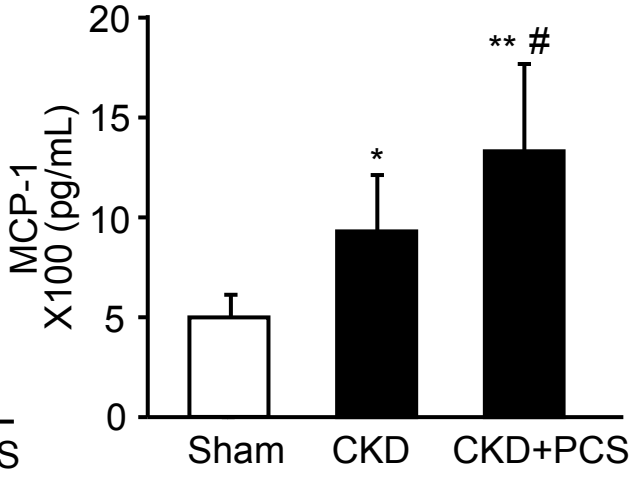
Osteopontin (aortic arch homogenate)



ALP activity (aortic arch homogenate)



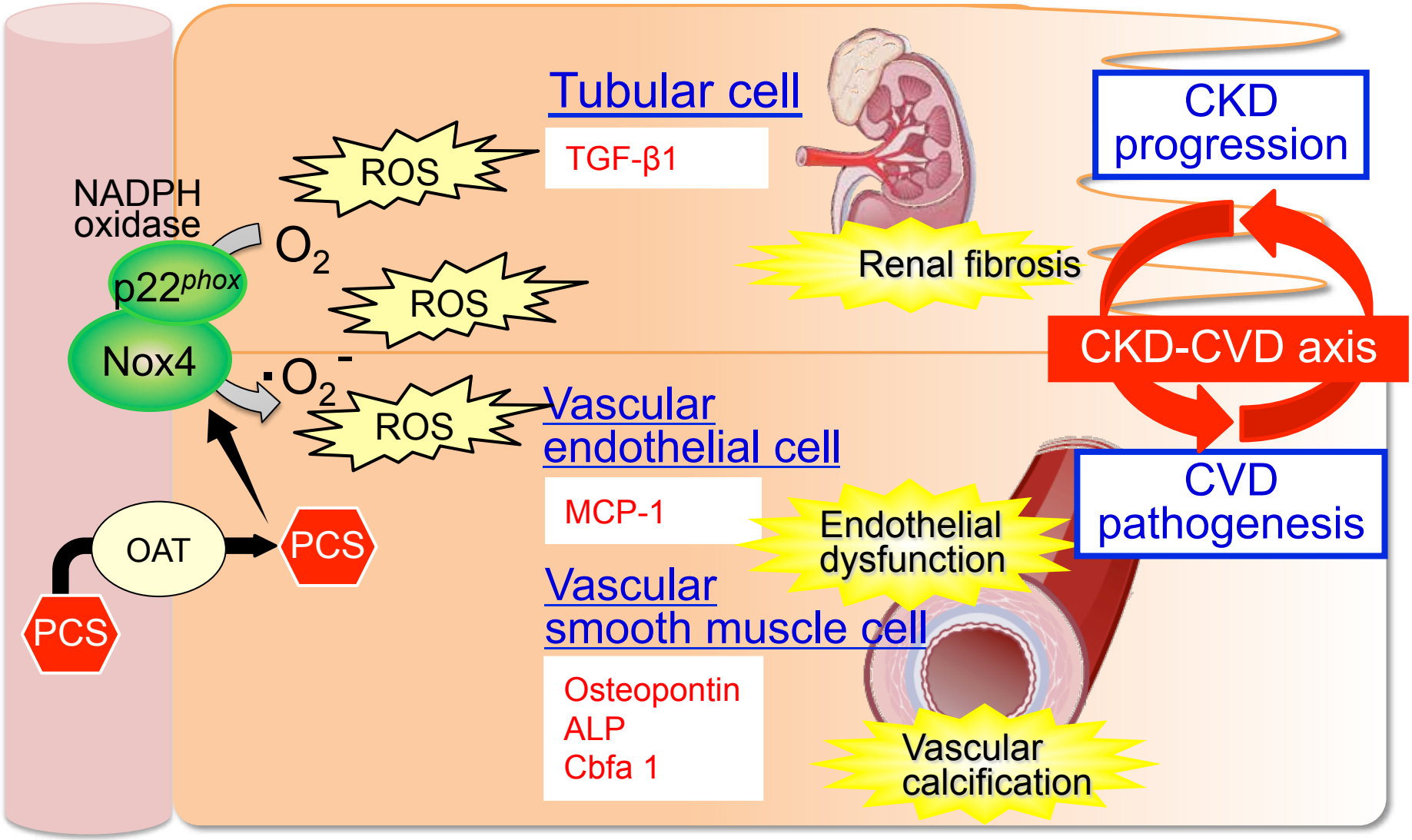
MCP-1 level (plasma)



In aortic arch, PCS-overload increases the expression of NADPH oxidase, osteopontin and ALP activity in addition to increasing plasma MCP-1 level.

Watanabe H et al. *Pharmacol Res Perspect.* 2014

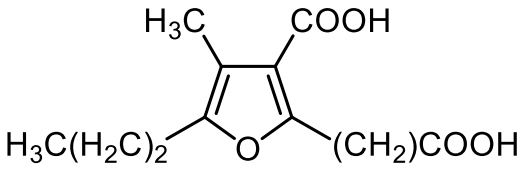
Intracellular accumulation of PCS *via* OATs could contribute to CKD-CVD axis

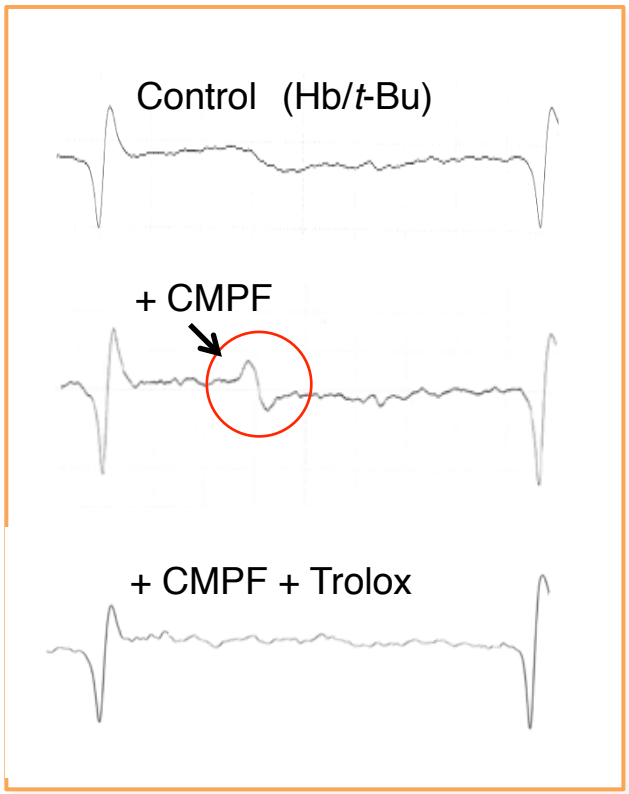


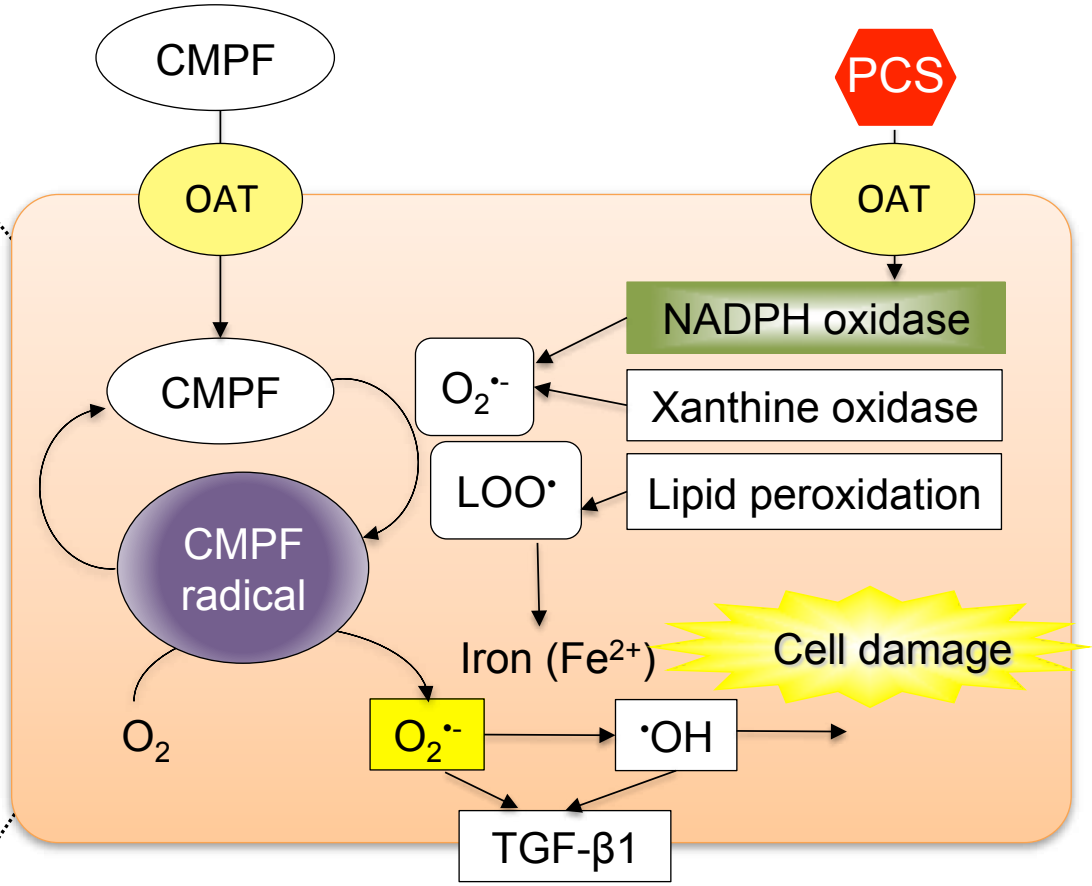
Watanabe H et al. *Kidney Int.* 2013
Watanabe H et al. *Pharmacol Res Perspect.* 2014

CMPF induces cell damage *via* generation of a radical intermediate

CMPF: 3-Carboxy-4-methyl-5-propyl-2-furanpropionate



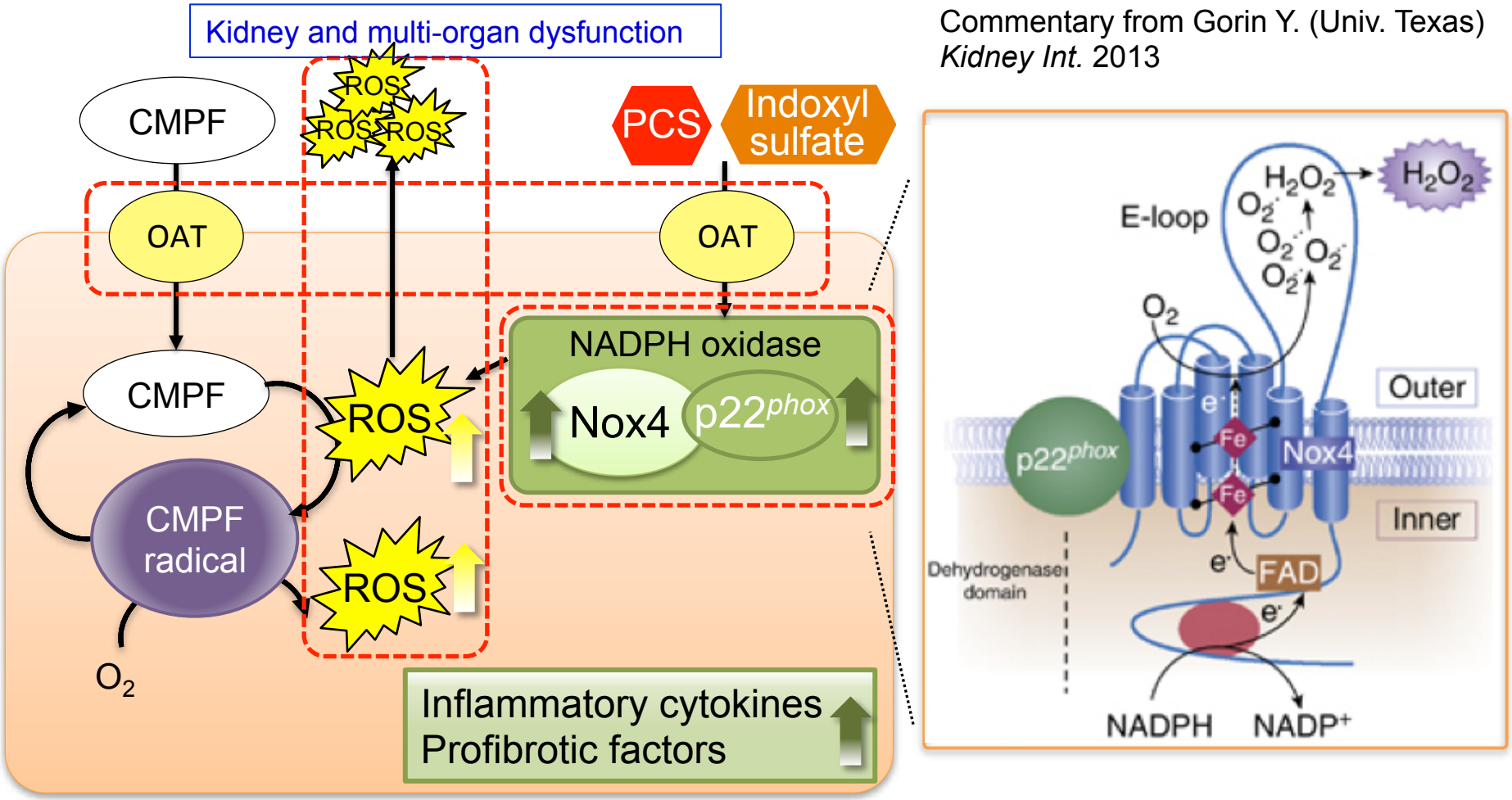




CMPF interacts with superoxide anion radicals ($O_2^{\bullet-}$) and peroxy radicals to produce CMPF radicals, which lead to cellular damage *via* overproduction of $O_2^{\bullet-}$.

Miyamoto Y et al. *Biochem Pharmacol.* 2012

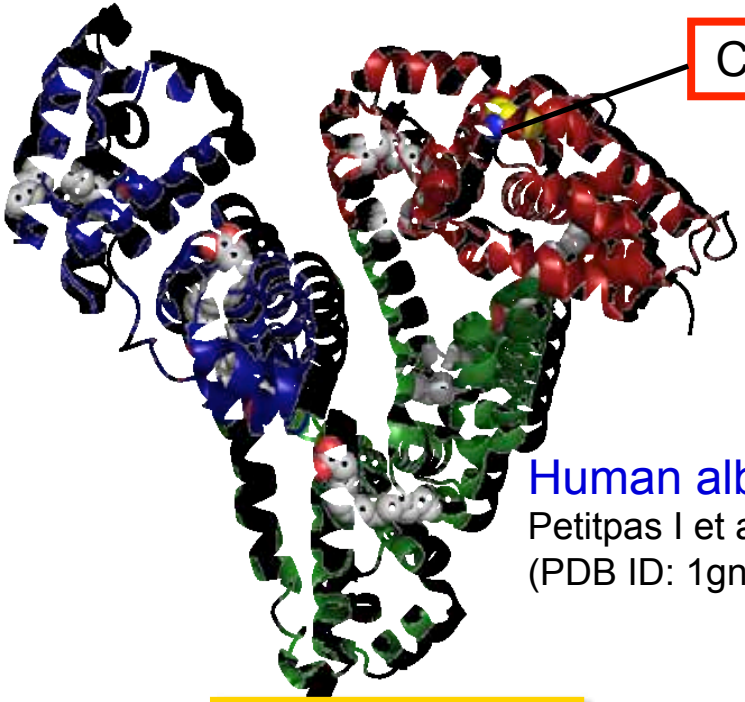
Potential therapeutic target for treatment of uremic toxicity associated to CKD



1. Cellular uptake pathway of uremic toxins, 2. NADPH oxidase, 3. Increased ROS

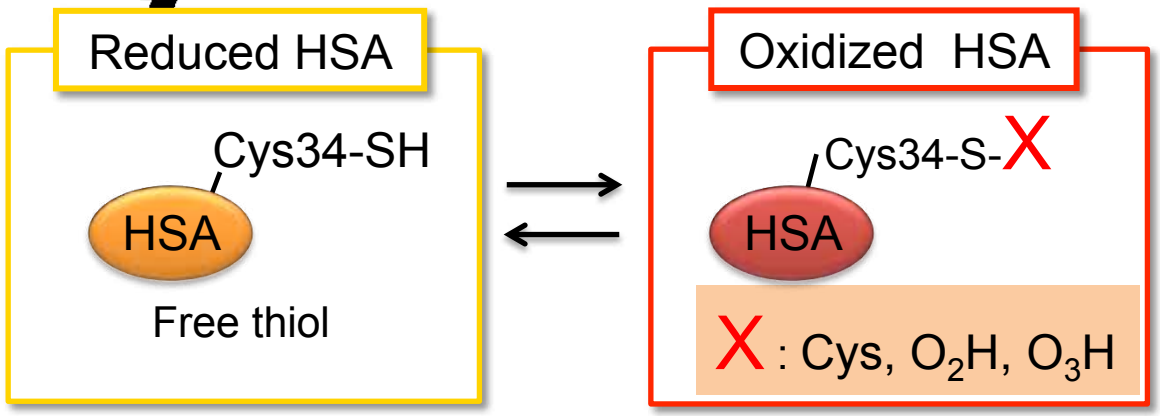
Our findings unraveled molecular mechanism of uremic toxin-induced progression of CKD and CVD.

New biomarker for CKD progression (Cys34-cysteinylated human albumin)



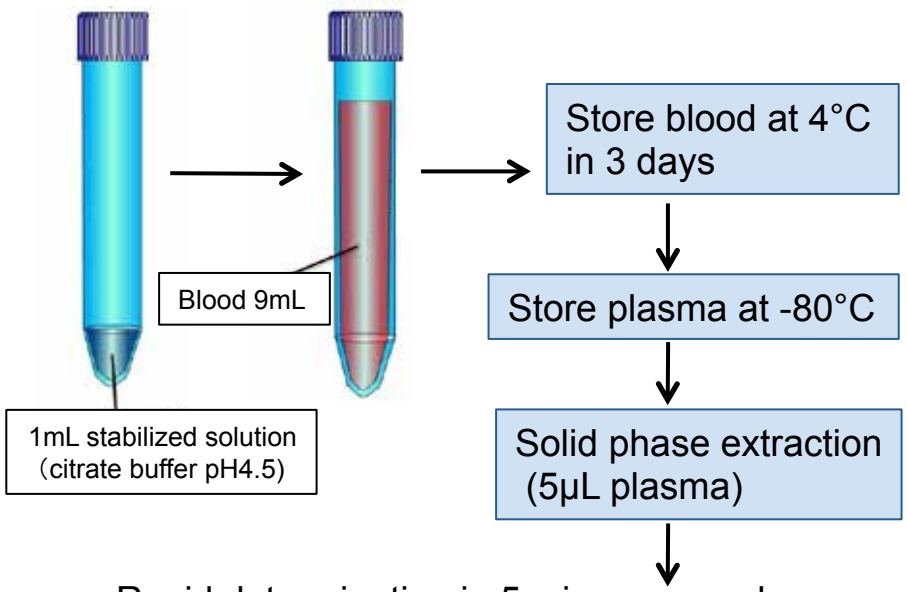
Cys34: only one free thiol group
(the main antioxidant site of HSA)

Human albumin (HSA)
Petitpas I et al. *J Mol Biol.* 2001
(PDB ID: 1gni)



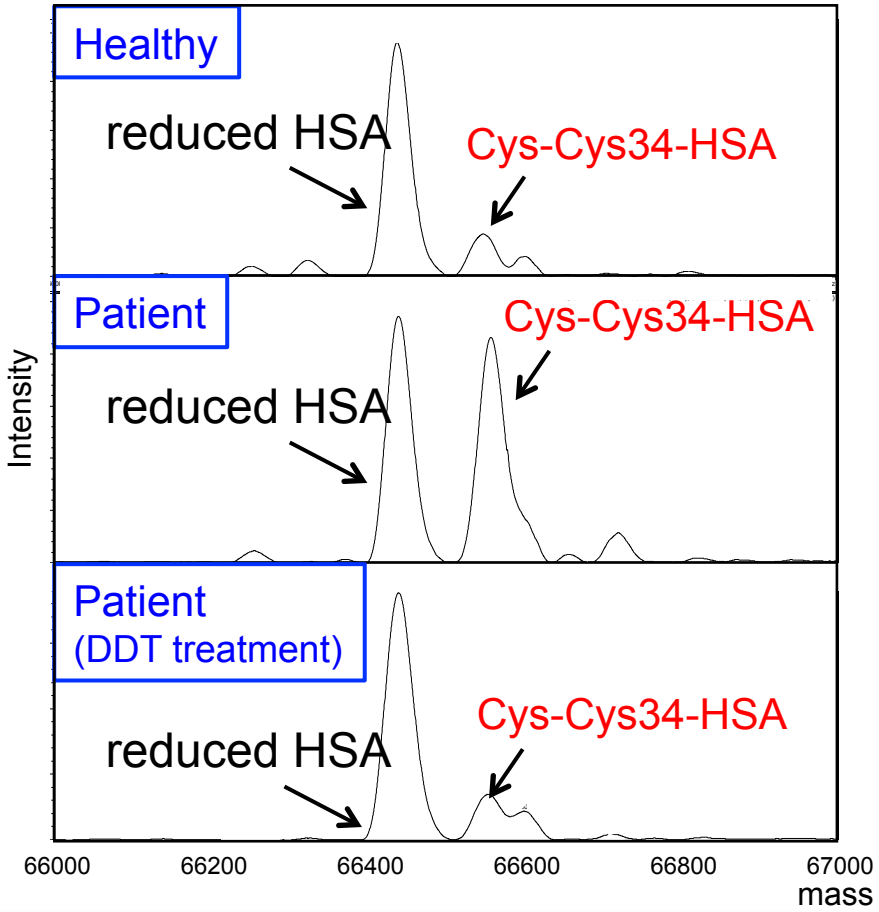
Anraku M et al. *Kidney Int.* 2004
Anraku M et al *BBA* 2013

Detection of redox change of Cys34 by using ESI-TOFMS



Rapid determination in 5 min per sample

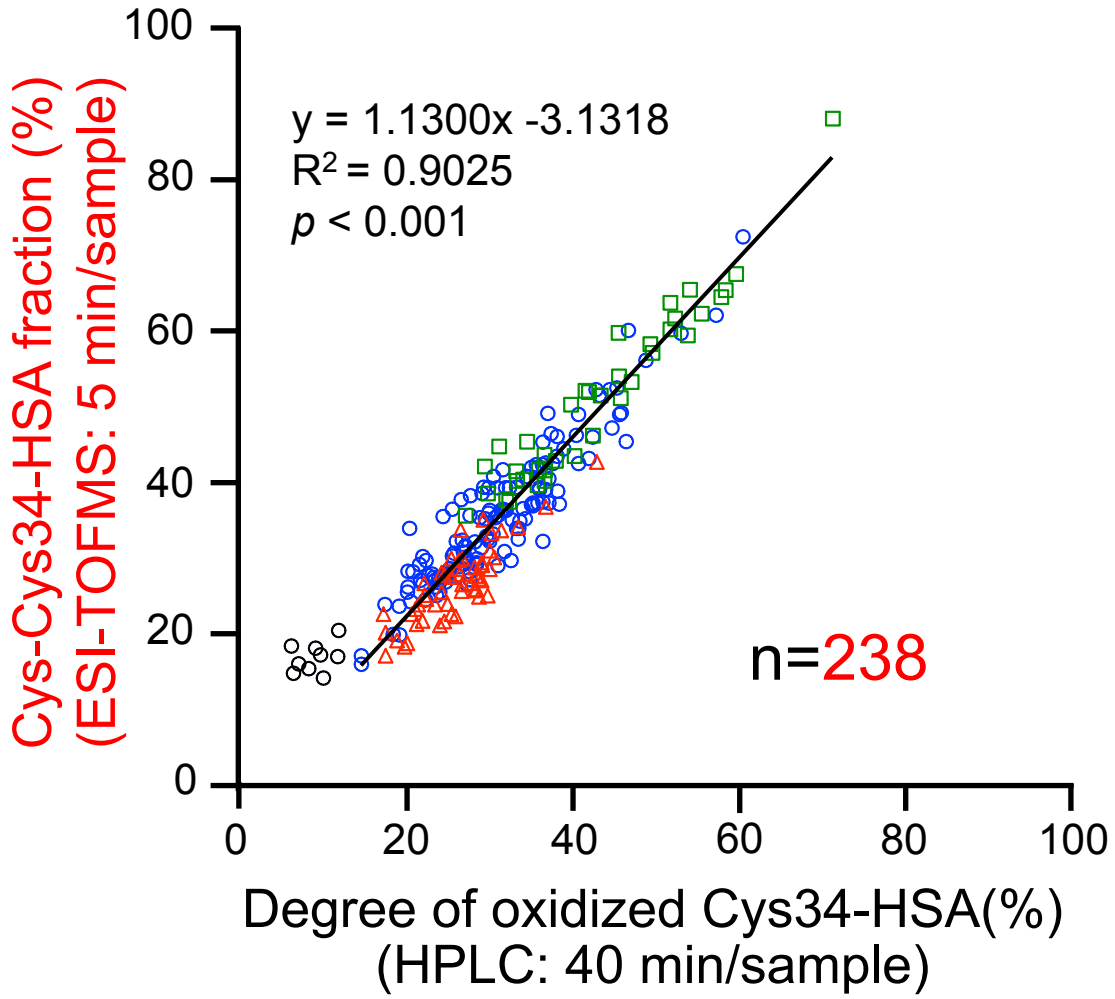
ESI-TOFMS specrta of HSA from healthy and patients



Major oxidized form is a disulfide-bonded Cys at Cys34 of HSA (Cys-Cys34-HSA)

Nagumo K et al. *PIOS ONE* 2014

Cys-Cys34-HSA fraction measured by using ESI-TOFMS is a new biomarker for oxidative stress

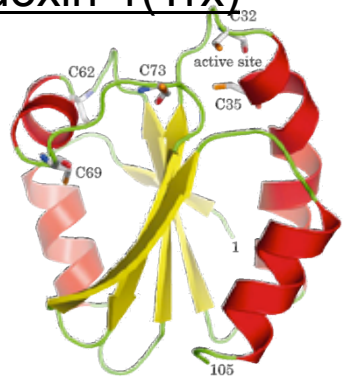


This system is a suitable analytical method for rapid and sensitive clinical laboratory analysis.

Nagumo K et al. *PIOS ONE* 2014

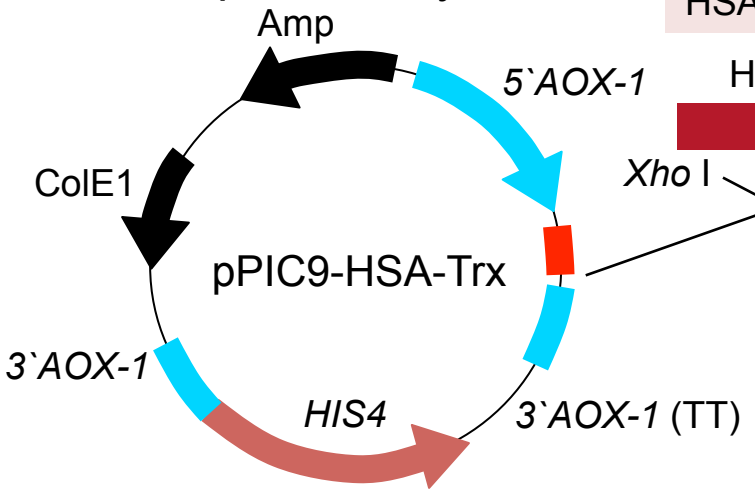
Novel anti-oxidative and anti-inflammatory agent albumin (HSA)-thioredoxin (Trx) fusion

Thioredoxin-1(Trx)

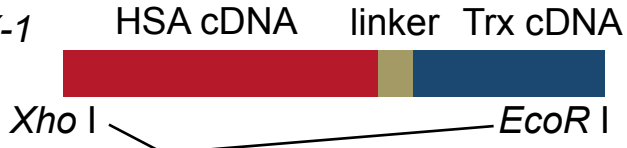


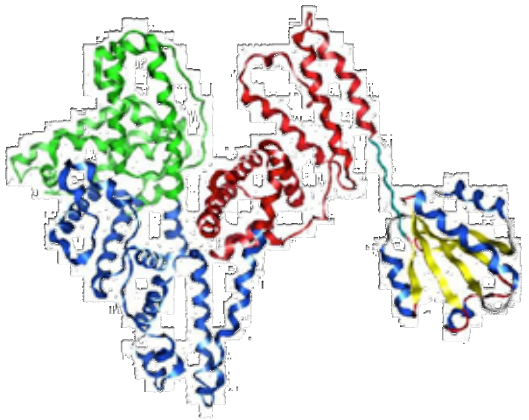
- ✓ anti-oxidative action
- ✓ anti-inflammatory action
- ✓ anti-apoptotic action
- ✓ plasma half-life ($t_{1/2}$) in mouse: 1 hr

Pichia expression system



HSA-Trx Fusion cDNA

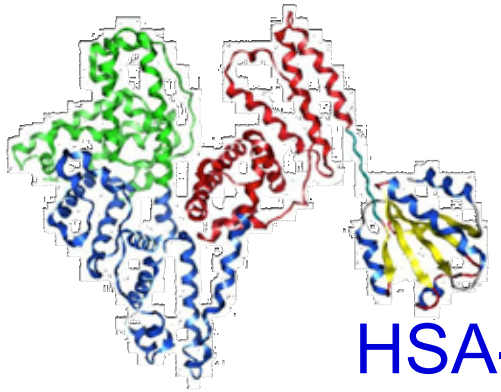




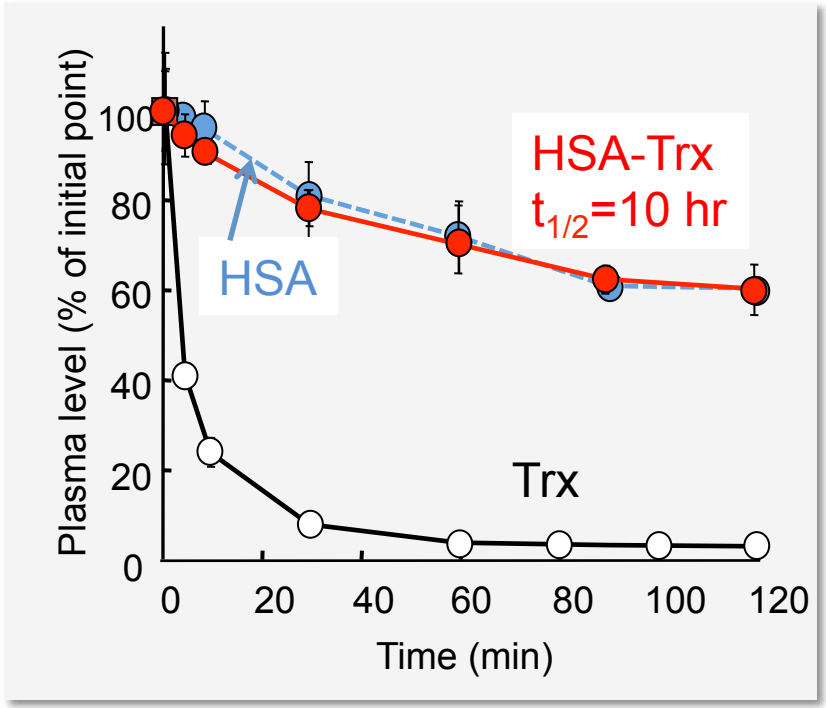
HSA-Trx
(image view)

Ikuta S et al. *J Control Release* 2010, Furukawa M et al. *J Control Release* 2011, Tanaka R et al. *JPET* 2013, Kodama A et al. *Kidney Int.* 2013, Kodama A et al. *BBA.* 2014, Tanaka R et al. *Mol Pharm.* 2014

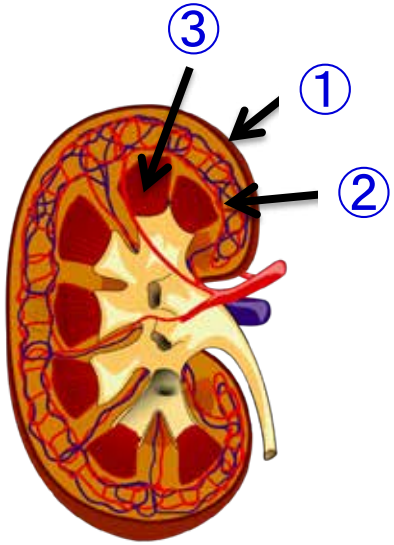
Plasma level-time curve and renal localization of HSA-Trx

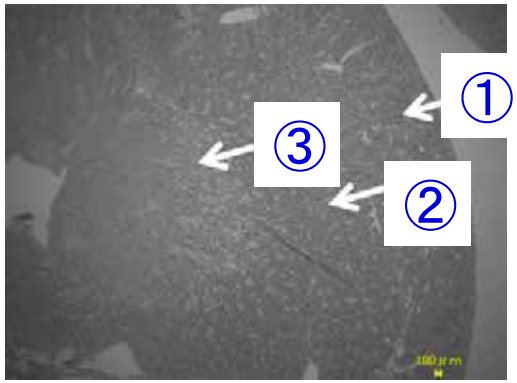


HSA-Trx

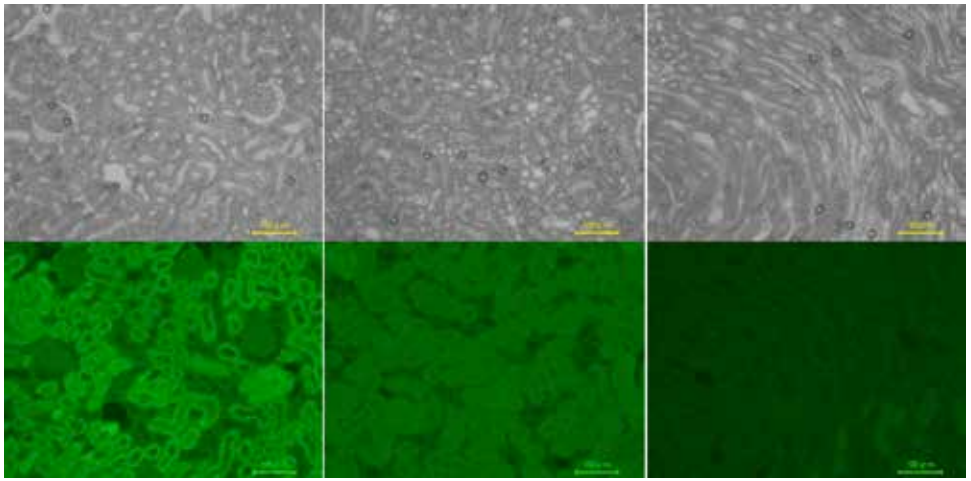


Ikuta S et al. *J Control Release* 2010,
Furukawa M et al. *J Control Release* 2011





①Cortex ②Cortico-medullary boundary zone ③Medulla



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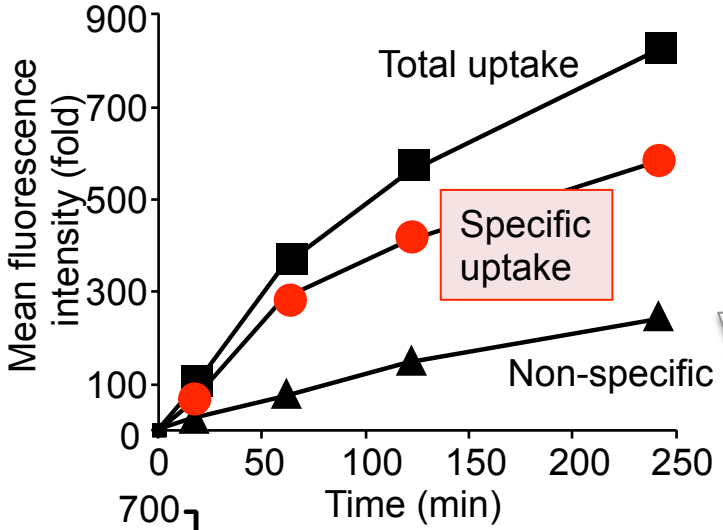
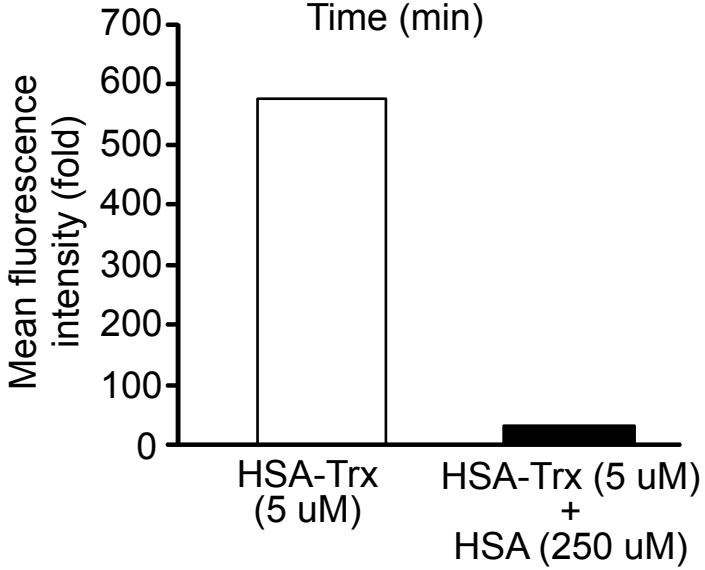
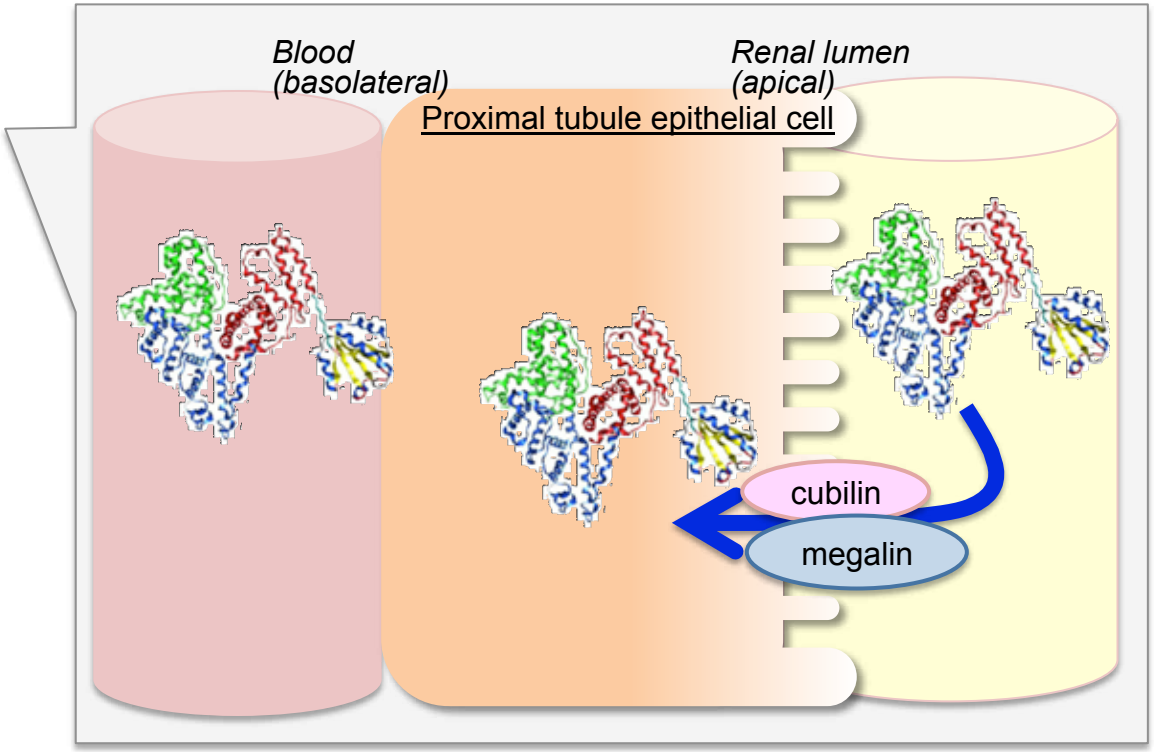
Uptake of HSA-Trx by human proximal tubular cell (HK-2) *via* megalin/cubilin-mediated endocytic pathway







HSA-Trx can act not only in extracellular compartment but also inside the proximal tubular cells.

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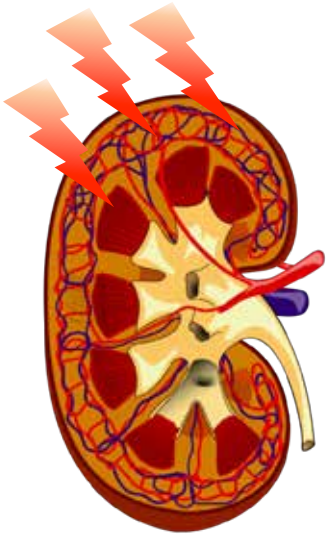
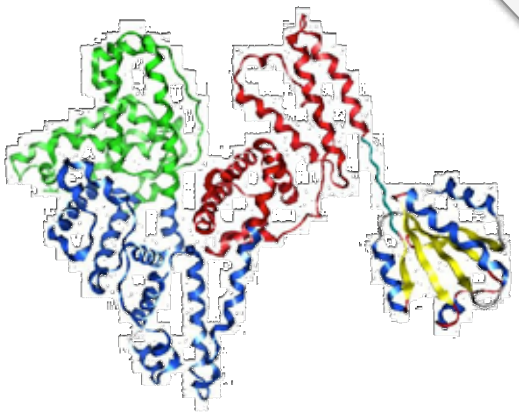
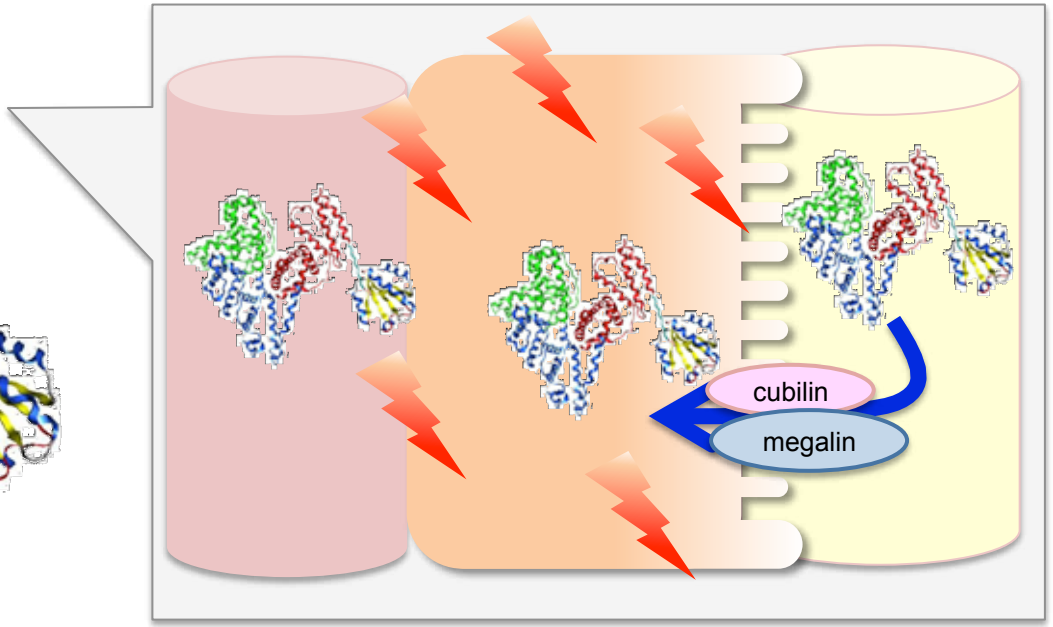
Renoprotective effect of HSA-Trx on nephropathy model animals

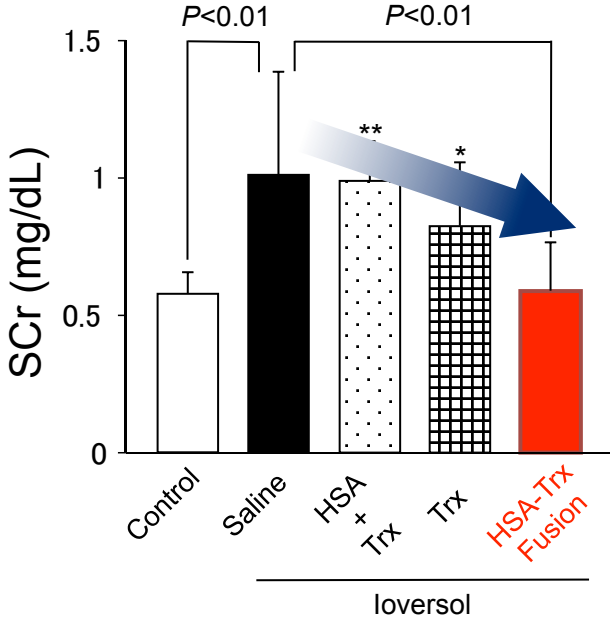


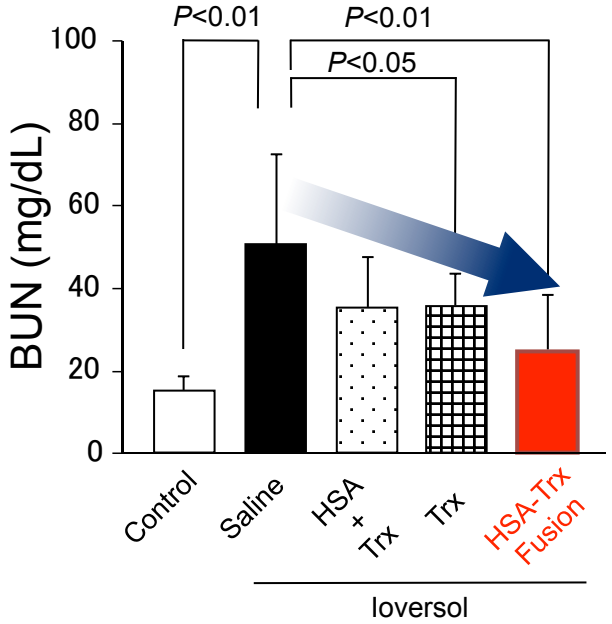
1.Contrast-induced nephropathy
2.Cisplatin-induced nephropathy
3.Rhabdomyolysis nephropathy

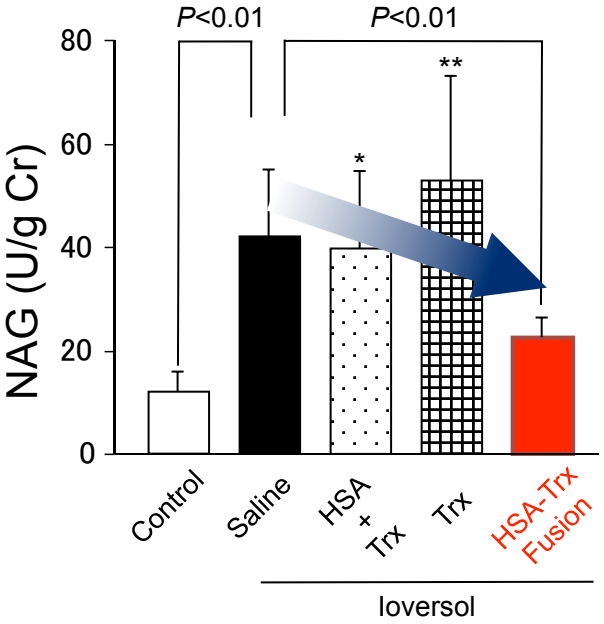


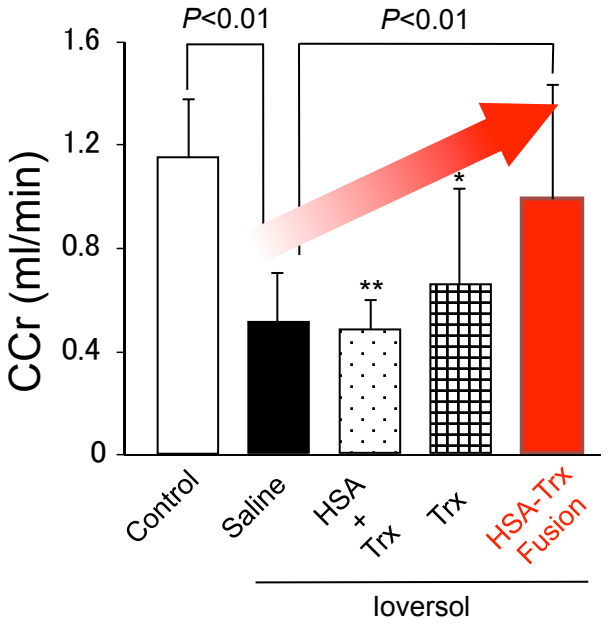


Renoprotective effect of HSA-Trx on contrast-induced nephropathy

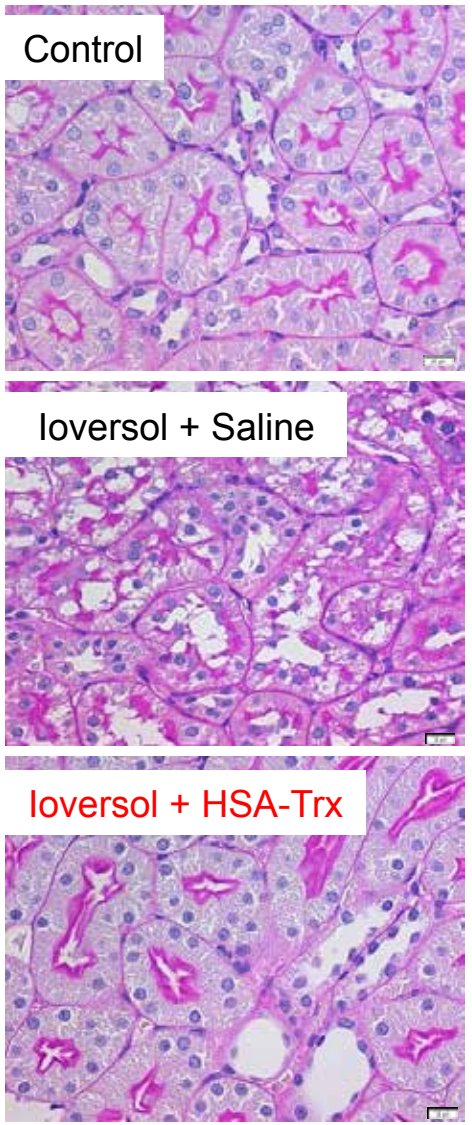








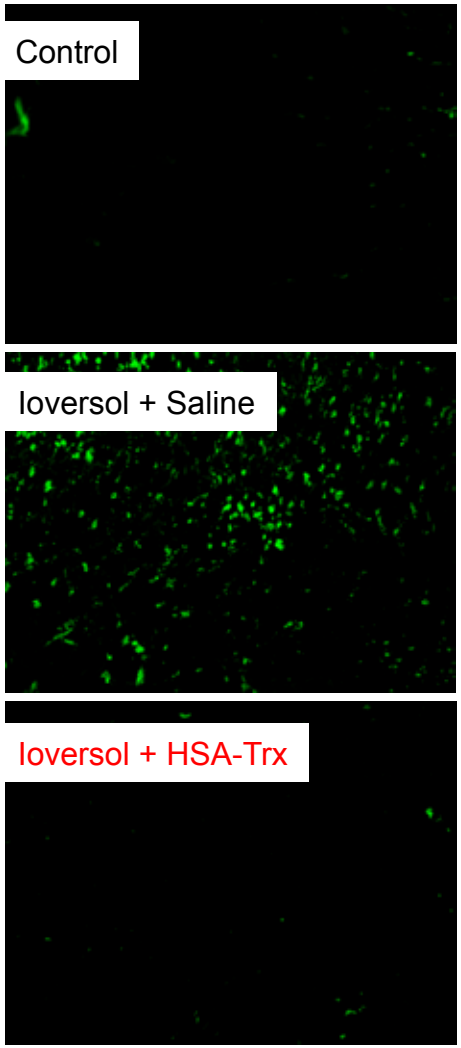
PAS staining



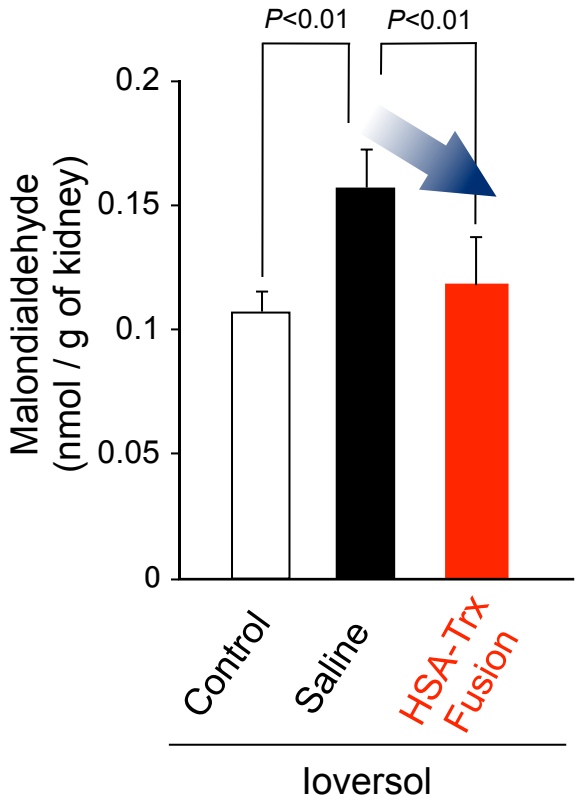
Kodama A et al. *Kidney Int.* 2013

The redox effect and anti-apoptotic effect of HSA-Trx on contrast-induced nephropathy

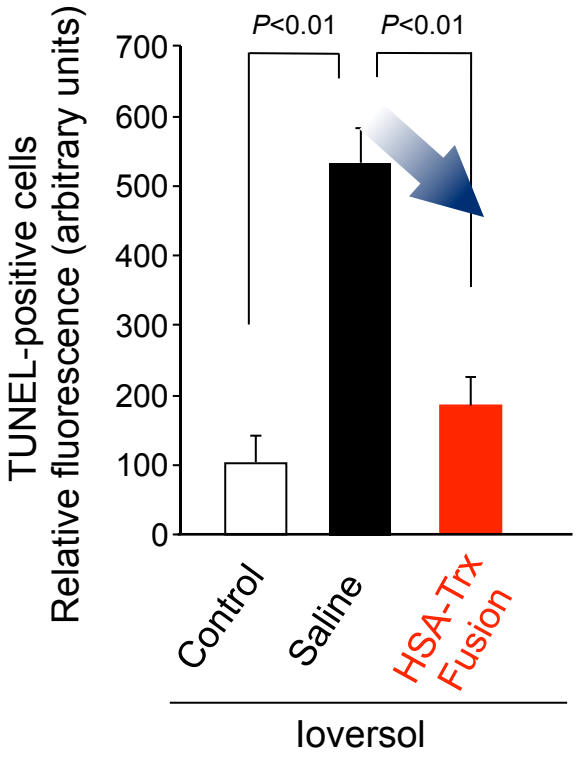
8-OHdG
(Oxidative DNA damage)



Malondialdehyde
(Oxidative lipid damage)



TUNEL positive cells



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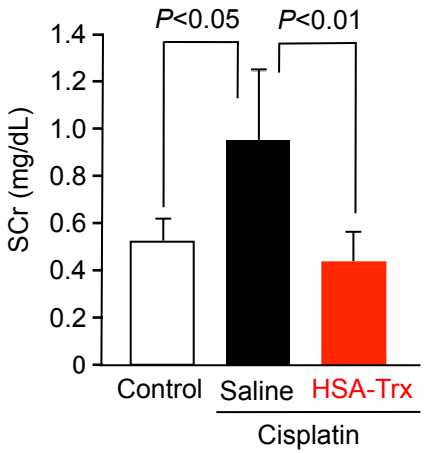
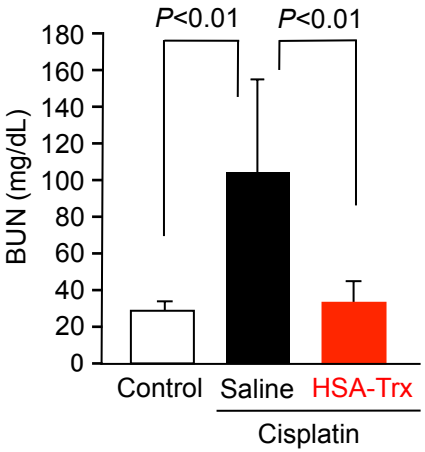
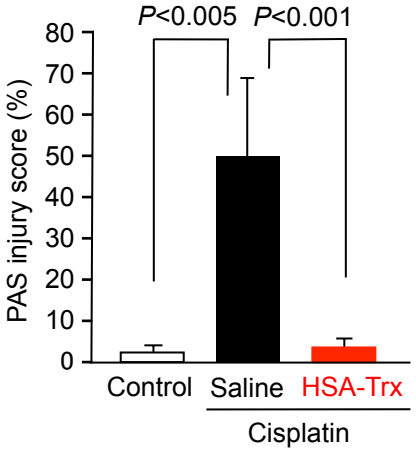
HSA-Trx prevents cisplatin-induced nephropathy

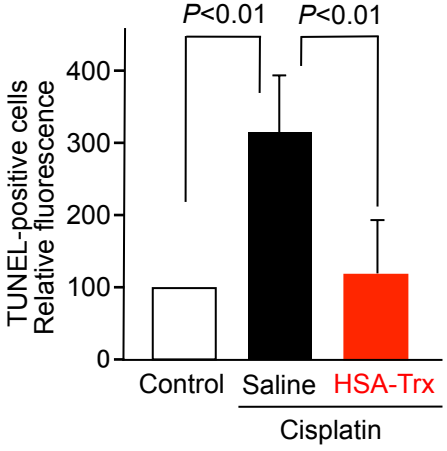
Renal inury



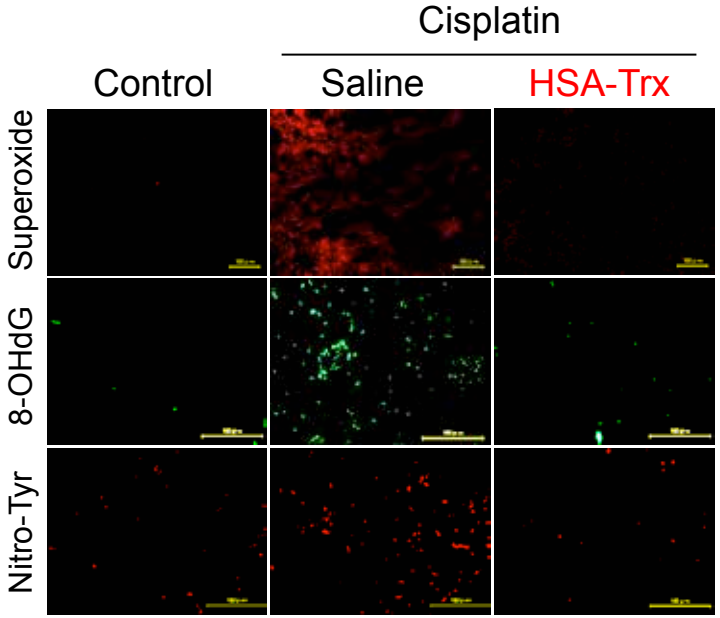




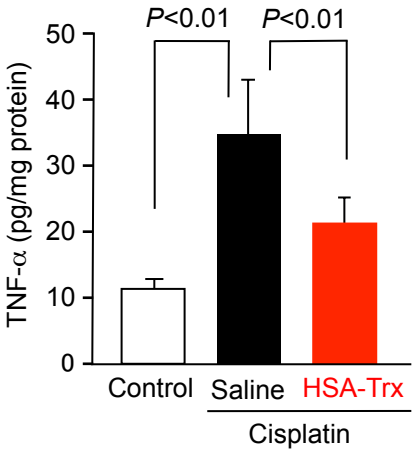
Renal Apoptosis

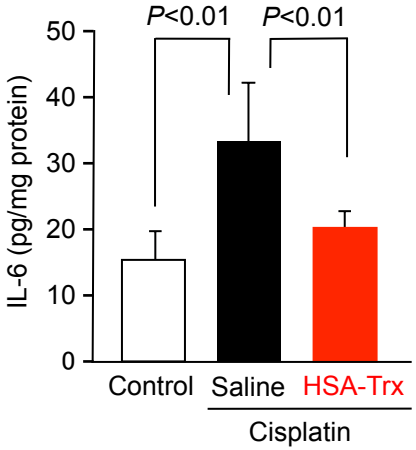


Renal oxidative stress



Renal inflammation





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Summary

Therapeutic approaches to progression of CKD and CVD

1. Molecular mechanism of uremic toxin-induced tubular and vascular damage

Miyamoto Y et al. *FEBS Lett*. 2010
Miyamoto Y et al. *Nephrol Dial Transplant*. 2011
Miyamoto Y et al. *Ther Apher Dial*. 2011
Watanabe H et al. *J Pharm Sci*. 2011
Miyamoto Y et al. *Biochem Pharmacol*. 2012
Watanabe H et al. *Drug Metab Dispos*. 2012
Tanaka H et al. *Bone* 2013
Watanabe H et al. *Kidney Int*. 2013
Watanabe H et al. *Pharmacol Res Perspect*. 2014

CKD/Uremic toxicity and CVD
(Oxidative stress)

2. Biomarker identification

Nagumo K et al. *PIOS ONE* 2014

3. Therapeutic development

Tanaka R et al. *JPET* 2013
Kodama A et al. *Kidney Int.* 2013
Kodama A et al. *BBA* 2014
Tanaka R et al. *Mol Pharm.* 2014

The findings provide new insight into the pathogenesis of CKD and lead to new therapeutic approaches to diagnosis and treatment of CKD.

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