Osteocrin, a bone-derived humoral factor, exerts a renoprotective role in ischemia–reperfusion injury in mice

Yoshihiko Nishiguchi1,* , Yusuke Hata1,* , Ryosuke Date1,* , Daisuke Fujimoto1 , Shuro Umemoto1 , Tomoko Kanki1 , Hideki Yokoi2 , Keita P. Mori2 , Takaya Handa2 , Haruko Watanabe-Takano3 , Yugo Kanai4 , Akihiro Yasoda5 , Yuichiro Izumi1 , Yutaka Kakizoe1 , Naoki Mochizuki3 , Masashi Mukoyama1 and Takashihe Kuwabara1

1Department of Nephrology, Kumamoto University Graduate School of Medical Sciences, Kumamoto, Japan, 2Department of Nephrology, Kyoto University Graduate School of Medicine, Kyoto, Japan, 3Department of Cell Biology, National Cerebral and Cardiovascular Center Research Institute, Osaka, Japan, 4Department of Diabetes Mellitus and Endocrinology, Osaka Red Cross Hospital, Osaka, Japan and 5Clinical Research Center, National Hospital Organization Kyoto Medical Center, Kyoto, Japan

*These authors contributed equally to this work.
Correspondence to: Takashihe Kuwabara and Masashi Mukoyama; E-mail: ktakasea@kumamoto-u.ac.jp and mmuko@kumamoto-u.ac.jp

ABSTRACT

Background. Osteocrin (OSTN), a bone-derived humoral factor, was reported to act on heart and bone by potentiating the natriuretic peptide (NP) system. Ostn gene polymorphisms have been associated with renal function decline, but its pathophysiological role in the kidney remains unclear.

Methods. The role of endogenous OSTN was investigated using systemic Ostn-knockout (KO) mice. As a model for OSTN administration, liver-specific Ostn-overexpressing mice crossed with KO (KO-Tg) were generated. These mice were subjected to unilateral ischemia–reperfusion injury (IRI) and renal lesions after 21 days of insult were evaluated. A comprehensive analysis of the Wnt/β-catenin pathway was performed using a polymerase chain reaction (PCR) array. Reporter plasmid-transfected proximal tubular cells (NRK52E) were used to investigate the mechanism by which OSTN affects the pathway.

Results. After injury, KO mice showed marginal worsening of renal fibrosis compared with wild-type mice, with comparable renal atrophy. KO-Tg mice showed significantly ameliorated renal atrophy, fibrosis and tubular injury, together with reduced expressions of fibrosis- and inflammation-related genes. The PCR array showed that the activation of the Wnt/β-catenin pathway was attenuated in KO-Tg mice. The downstream targets Mmp7, Myc and Axin2 showed similar results. MMP7 and Wnt2 were induced in corticomedullary proximal tubules after injury, but not in KO-Tg. In NRK52E, OSTN significantly potentiated the inhibitory effects of NP on transforming growth factor β1–induced activation of the Wnt/β-catenin pathway, which was reproduced by a cyclic guanosine monophosphate analog.

Conclusions. Ectopic Ostn overexpression ameliorated subsequent renal injury following ischemia–reperfusion. OSTN could represent possible renoprotection in acute to chronic kidney disease transition, thus serving as a potential therapeutic strategy.

Keywords: AKI to CKD, ischemia–reperfusion, natriuretic peptides, osteocrin, Wnt/β-catenin pathway

INTRODUCTION

Acute kidney injury (AKI) is a common complication in critically ill patients, associated with high morbidity and mortality as well as longer hospital stay and high medical burden. Historically it had been assumed that individuals who survived an episode of AKI could fully recover their renal function, but growing evidence has shown that those survivors have a significant risk of developing progressive chronic kidney disease (CKD) and even end-stage kidney disease [1–4]. That is, AKI is not only life-threatening in the acute phase, but often leads to CKD. In fact, the high burden of CKD was concentrated in low-resource settings, while a high incidence of AKI was observed in such countries [4], suggesting that the AKI–CKD continuum has become an emerging issue globally.

Natriuretic peptides (NPs) consist of atrial natriuretic peptide (ANP), brain (or B-type) natriuretic peptide (BNP) and C-type natriuretic peptide (CNP). ANP and BNP secreted from the heart exert potent natriuretic, diuretic and vasodilating...
What is already known about this subject?

- Osteocrin (OSTN) reportedly exerted bone elongation and cardioprotection through antagonizing the natriuretic peptide (NP) receptor C, a clearance receptor for NPs.
- A genome-wide association study targeting 60,000 European patients revealed that SNPs of the Ostn gene were related to a risk for rapid decline of renal function.
- The role of OSTN in the kidney has not yet been clarified.

What this study adds?

- Ectopic liver-specific overexpression of Ostn, mimicking exogenous OSTN administration, attenuated acute kidney injury (AKI) to chronic kidney disease (CKD) progression in mice.
- Comprehensive analysis by a polymerase chain reaction array showed that the changes in the Wnt/β-catenin pathway and its target genes observed in atrophic kidneys were canceled in Ostn-overexpressing mice.
- These effects were suggested to be due to suppression of the Wnt/β-catenin pathway via the enhancement of cyclic guanosine monophosphate signaling by OSTN.

What impact this may have on practice or policy?

- OSTN could provide a potential therapeutic strategy against AKI–CKD progression.
- OSTN locally potentiates the action of NPs and thus can exert renoprotective effects with minimal systemic effects such as hypotension with respect to direct administration of NPs.
- Such mechanisms may in part resemble those of an angiotensin receptor–neprilysin inhibitor, a clinically used agent expected to have cardio renal protective effects.

Osteocrin (OSTN, also known as musculin) is a small secreted peptide cloned from bone and muscle complementary DNA (cDNA) libraries [6, 21, 22]. OSTN has a well-conserved homology to members of the NP family but has no natriuretic activity. OSTN has been shown to bind to NPR-C with a high affinity and specificity, thereby potentially antagonizing NP clearance and increasing the local availability of NPs [23, 24].

We recently reported that OSTN exerts bone elongation and cardioprotective actions in mice through antagonizing NPR-C [25, 26]. Of note, a genome-wide association study targeting 60,000 European patients found that single-nucleotide polymorphisms (SNPs) of the Ostn gene were related to a risk for rapid decline of renal function [27]. However, the functional role of OSTN in the kidney has remained obscure. In this study we aimed to elucidate the role of OSTN on renal pathophysiology using a rodent model of AKI–CKD transition.

Materials and Methods

For the full method, please refer to the supplementary material.

Animals

We generated systemic Ostn-knockout (KO) mice to investigate the role of endogenous OSTN in the kidney. We next generated Ostn-transgenic mice with elevated plasma levels of OSTN due to liver-specific Ostn overexpression and then mated them with KO (KO-Tg) [25, 26]. Detailed information of Ostn-KO-Tg was described previously [25].

Experimental design in vivo and renal ischemia–reperfusion injury (IRI)

The protocol to develop AKI Ostn-transgenic mice CKD and AKI models are described in the supplementary material. All experiments were conducted in accordance with the Guidelines for Animal Research Committee of Kumamoto University (certification A30-013).
FIGURE 1: Plasma OSTN, urinary cGMP levels and renal weight among genotypes. (a) Plasma OSTN and (b) urinary cGMP were measured by CLEIA and ELISA, respectively. n = 4–5. (c) Assessment of renal atrophy and the renal weight among genotypes. n = 7–8. Bars show mean ± SD. **P < 0.01, KO versus KO-Tg; ††P < 0.01, WT versus KO-Tg.

Measurement of plasma OSTN

Measurement of mouse plasma OSTN was performed as we previously reported [25]. Plasma OSTN concentrations were measured by sandwich chemiluminescence enzyme immunoassay (CLEIA), modifying the previously reported protocol for mouse ANP CLEIA [28].

Histology and immunohistochemical staining

Azan staining was carried out using kidney sections (thickness 4 μm) fixed with 4% buffered paraformaldehyde. The protocol and information of primary antibodies for immunohistochemistry are described in the supplementary material. Eight randomly selected fields per section from each mouse were recorded using a BZ-X700 All-in-One Fluorescence Microscope (Keyence, Osaka, Japan) and the fibrotic areas were quantified by Hybrid Cell Count software (Keyence). We calculated the fibrotic area relative to the total area of the field as a percentage.

Real-time quantitative PCR (qPCR)

Total RNA was extracted from mouse kidneys and cDNA in each sample was synthesized. TaqMan real-time PCR was performed by Quantstudio 5 (Applied Biosystems, Waltham, MA, USA) (see Supplementary data, Table S1 for primer and probe sequences). Expression levels of all genes were normalized by Gapdh (internal control) levels.

PCR array and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway analysis

An RT2 Profiler PCR array specific for the Wnt signaling pathway and Wnt signaling target components was used to assess transcript levels in the kidneys. The arrays were run on a Viia7 Real-Time PCR System (Applied Biosystems). KEGG (www.genome.jp/kegg/pathway.html) pathway analysis was conducted to identify the pivotal part in the Wnt pathway.

Cell culture and luciferase assay

Rat renal proximal tubular cell line (NRK52E) was used for in vitro study. The Wnt/β-catenin transcriptional activity was evaluated using the TopFlash luciferase reporter assay. Details are provided in the supplementary material.

Measurement of cGMP levels in urine and cultured-cell lysates

The cGMP was measured using the Cyclic GMP ELISA Kit (Cayman Chemical, Ann Arbor, MI, USA) following the manufacturer's instructions.

Statistical analyses

Data are expressed as mean ± standard deviation (SD) and standard error of the mean (SEM) in in vivo and in vitro studies, respectively. Differences between multiple groups were assessed by one-way factorial analysis of variance with a post hoc test. Comparison between two groups was carried out by an unpaired Student's t-test. P-values <0.05 were regarded as statistically significant.

RESULTS

Overexpression of Ostn ameliorated renal atrophy and fibrosis

To investigate both the endogenous and exogenous roles of OSTN in the kidney, we conducted studies using Ostn-KO and KO-Tg mice. Prior to the kidney injury experiment, we measured plasma OSTN and urine cGMP levels in wild-type (WT), KO and KO-Tg mice. Plasma OSTN in KO-Tg mice showed 35.2 ± 6.9 ng/mL, whereas those in WT and KO mice were below the detection limit (Figure 1a), which seems to be consistent with our previous reports [25, 26]. Urine cGMP levels are comparable between WT and KO, although those of KO-Tg were higher (Figure 1b).

To prepare an AKI–CKD model, we performed unilateral IRI to these mice and collected tissue samples 3 weeks after IRI. The kidneys subjected to IRI had severe atrophy (by ~75% reduction in weight of the contralateral kidney) in WT and also in KO mice (Figure 1c). Histological examination with Azan staining revealed remarkable fibrosis in the injured kidney from both WT and KO, especially at the corticomedullary region and cortex (Figure 2a). Similar results
were also confirmed by α-smooth muscle actin (α-SMA) staining (Supplementary data, Figure S1). Although KO mice showed marginal worsening of kidney fibrosis compared with WT mice, it was not statistically significant (Figure 2b). In KO-Tg mice, on the other hand, such atrophy of the injured kidney was significantly mitigated (by an ~50% reduction in weight; significantly heavier than those from WT and KO mice) (Figure 1c). Likewise, fibrotic changes were significantly less in the kidney from KO-Tg compared with WT and KO mice (Figure 2a); the quantification of the fibrotic area indicated that the area was about half in KO-Tg mice (Figure 2b).

In order to further evaluate the acute phase, a similar study was conducted in the AKI model by IRI following unilateral nephrectomy. The results showed that (α-SMA), transforming growth factor-β (TGF-β) and neutrophil gelatinase-associated lipocalin (NGAL) expression in the kidneys tended to be lower in KO-Tg mice, but renal function was comparable between genotypes (Supplementary data, Figure S2).

Upregulated gene expressions of proinflammatory, profibrotic and tubular injury markers were suppressed by Ostn overexpression

Next we investigated the gene expressions of proinflammatory, profibrotic and tubular injury markers in the atrophic kidney by real-time qPCR. In KO mice, interleukin (IL)-1β,
FIGURE 3: Gene expressions of proinflammatory, profibrotic and tubular injury markers in the kidney. The gene expressions in the contralateral and IRI-induced atrophic kidneys by TaqMan real-time qPCR. As all genes were significantly different between contralateral and IRI within the group, the symbols were omitted. Bars show mean ± SD. *P < 0.05, **P < 0.01, KO versus KO-Tg; †P < 0.05, †† P < 0.01, WT versus KO-Tg. n = 6–12.

α-SMA and collagen type I alpha1 (COL1A1) messenger RNA (mRNA) expressions tended to be enhanced compared with WT mice (Figure 3). In KO-Tg mice, on the other hand, not only these genes but TGF-β1, fibronectin 1 (Fn1) and NGAL mRNA expression showed less upregulation compared with WT or KO mice (Figure 3). Thus it was suggested that the endogenous role of OSTN against kidney inflammation and fibrosis after IRI appeared limited, but increased circulating levels of OSTN by Ostn overexpression could ameliorate the fibrotic changes as well as the inflammation- and fibrosis-related gene upregulation in the injured kidney. Furthermore, we examined the mRNA expression of NPR-A, B and C in the kidney tissue and found that NPR-A and NPR-B did not differ significantly by IRI and genotype, while NPR-C was upregulated by IRI, and this increase was clearly canceled in KO-Tg mice (Supplementary data, Figure S3). Taken together with the aforementioned results of elevated urinary cGMP in KO-Tg mice (Figure 1b), it is possible that OSTN overexpressed in the liver exerted its effect by acting locally in the kidney.

Alterations in the Wnt/β-catenin pathway and its target genes observed in KO fibrotic kidneys were alleviated in KO-Tg mice

In order to investigate the mechanism of OSTN-mediated suppression of renal fibrosis, we performed a comprehensive analysis of the Wingless-related integration site (Wnt) pathway, which has been reported to play an important role in fibrosis during AKI–CKD transition [29, 30], using a PCR array (Figure 4 and Supplementary data, Figure S4). In the planer cell polarity (PCP) and Wnt/calcium pathways, we found only unremarkable changes between the contralateral and fibrotic kidneys after IRI in KO and KO-Tg mice. In the Wnt/β-catenin pathway, on the other hand, we identified several genes and their target genes that were markedly altered. Of note, major changes in KO-Tg relative to KO mice included restoration of the decrease in negative regulators as well as reduction of the increase in the central axis genes of the Wnt/β-catenin pathway. Real-time qPCR recapitulated the alterations of some of the major genes downstream of this pathway (Figure 5). Also, we also investigated the PCP pathway and Wnt/calcium pathway by qPCR (Supplementary data, Figure S5). These changes were nearly unchanged or obviously much weaker than the Wnt/β-catenin pathway shown in Figure 5. Note that there was no difference between WT and KO mice. The Wnt ligands Wnt2 and Wnt16 also exhibited changes parallel to the Wnt/β-catenin target genes (Figure 5).

To examine the localization of matrix metalloproteinase-7 (MMP7) and Wnt2, immunohistochemical staining was performed (Supplementary data, Figure S6). As a result, Wnt2 and MMP7 seem to be upregulated in aquaporin 1-positive proximal tubular epithelial cells of the corticomedullary junction in the IRI-induced fibrotic kidney. Such induction was virtually absent in the injured kidney of KO-Tg mice (data not shown). However, we have not been able to present these co-stainings and care should be taken in interpreting them.

OSTN enhanced ANP-induced suppression of the Wnt/β-catenin pathway

So far, in vivo experiments have shown that the increased circulating levels of OSTN appear to suppress the upregulation of the Wnt/β-catenin pathway and alleviate the progression of renal fibrosis. Therefore we then investigated whether the administration of OSTN in vitro suppresses the Wnt/β-catenin pathway using the proximal tubular cell line NRK52E. Prior to this experiment, we confirmed the mRNA expression of NPR-A, NPR-B and NPR-C in NRK52E (Supplementary data,
Figure 4: KEGG pathway of Wnt signaling in IRI kidney. The black and gray gene columns indicate that the genes with a >2-fold increase or less than half decrease in IRI in KO, respectively, and that the change was in the direction of recovery in KO-Tg. Figure was re-created and modified based on the KEGG pathway.

Figure S7). TopFlash reporter plasmid-transfected NRK52E cells were treated with TGFβ1, ANP, OSTN and 8-Br-cGMP (Figure 6a). The luciferase assay showed that the addition of OSTN significantly potentiated the inhibitory effect of ANP on the activation of the Wnt/β-catenin pathway, which was induced by TGF-β1. Note that cGMP levels of cell lysates were amplified by ANP and OSTN treatment (Figure 6b). Treatment with 8-Br-cGMP, a cGMP analog, revealed a similar inhibitory effect, suggesting that the enhancement of OSTN in ANP action could be mediated by increasing cGMP levels within the cells.

DISCUSSION
In the present study, we revealed that the ectopic overexpression of Ostn with potentially increased circulating OSTN levels resulted in ameliorated renal injury after IRI, suggesting that OSTN administration could exert a possible renoprotective action. Although it has been reported that SNPs of Ostn are associated with the risk of rapid decline of renal function [27], the details of its causal relationship remain unknown. In this study we showed for the first time the inhibitory role of OSTN in AKI–CKD transition in mice.

As demonstrated in a systematic review and meta-analysis [31], an unequivocal association of AKI with subsequent CKD is a great problem, leading to end-stage renal failure and death. At present, however, therapeutic interventions available to halt this transition are very limited and basic and translational research is being actively conducted to understand molecular and cellular mechanisms underlying the pathogenesis. The Wnt/β-catenin pathway is thought to play an important role in the progression toward CKD [29, 30]. In the present study, we showed that Ostn overexpression suppressed activation of the Wnt/β-catenin pathway and mitigated renal fibrosis after IRI. We further showed that the protective effect of OSTN could be at least partly exerted through inhibition of the Wnt/β-catenin pathway by enhancing NP signaling. It has been reported that the anti-tumor effect of sulindac could be mediated by suppressing the Wnt/β-catenin pathway through increasing cGMP and protein kinase G (PKG) activities [32]. In the present study, cGMP was also found to be increased by KO-Tg mice in urine and by co-incubation of ANP and OSTN in cultured proximal tubular cells. The distal tubules and collecting ducts are the primary sites of the natriuretic action of ANP, but the proximal tubules, the main target in IRI, are also important in ANP actions [33–36]. In fact, OSTN administration to proximal tubular cells expressing NPR-A and NPR-C could potentiate the ANP’s inhibitory effect of the Wnt/β-catenin pathway, the action of which was recapitulated by addition of a cGMP analog (Figure 6). It is difficult to answer conclusively why OSTN is important for the canonical pathway within the Wnt pathway, but it is possible that ANP inhibits the β-catenin pathway in a manner that competes with Wnt ligands for Frizzled receptors [37]. It should be noted that the present study does not show direct evidence of fibrosis...
in proximal tubular cells. Crosstalk between tubular cells and fibroblasts is reportedly important for fibrosis, indicating that fibroblasts may be involved [38]. However, it is a limitation that the expression of NPR1 and 3, the receptors for ANP and OSTN, is poor in fibroblast cell lines and has not been investigated (Supplementary data, Figure S7).

The present study revealed that systemic Ostn KO mice showed marginal worsening of renal fibrosis but largely resulted in similar deterioration in various pathologies and gene expressions as compared to WT mice. Under normal conditions, Ostn expression is almost undetectable in the kidney, and osteoblasts are the main source of production [21]. The endocrine role of bone-derived OSTN remains unknown and has to be elucidated, but it could act locally in a paracrine fashion in tissues other than bone. In fact, in our previous reports and in the present study, plasma OSTN levels in both WT and KO mice were below the detection limit, while a clear elevation in blood levels was observed in KO-Tg mice. The blood concentration in overexpressing mice greatly exceeds the level working for paracrine, suggesting that it is protective in pharmacological dose. In addition, urinary cGMP, a marker for the action of NPs locally in the kidney, was similar between WT and KO mice and increased in KO-Tg mice, which was thought to support this finding. However, we have confirmed that Ostn is expressed in damaged proximal tubules after IRI (data not shown), thus we cannot deny the possibility that OSTN produced locally may play a role in less-damaged models. Besides, this model was limited by the fact that the degree of renal atrophy that could be analyzed was 3 weeks after IRI. It would be desirable to examine in a model that allows longer-term observation. It has been reported that the addition of 1,25-dihydroxyvitamin D3 to osteoblasts suppresses Ostn expression via the vitamin D receptor [21]. Therefore it is possible that decreased vitamin D3 activity during renal failure might lead to the induction of Ostn re-expression.

Analysis of Ostn KO mice revealed that endogenous OSTN promotes long-axis and short-axis bone growth and that the site of expression coincides with the site where extension is stimulated by loading in the distal bone [39]. In recent years, it has been demonstrated that the prognosis of patients with CKD is related to their physical activities, and the importance of renal rehabilitation has been suggested [40]. In animal models of CKD, chronic exercise was shown to have a renoprotective effect [41]. Likewise, in clinical studies, a randomized controlled trial in patients with stage 3–4 CKD as well as in obese patients with CKD showed that exercise therapy can improve eGFR [42, 43]. In these circumstances, it might be possible that the increased secretion of OSTN, whose expression is enhanced by bone loading, could contribute to the renoprotective effect upon exercise. To develop the assay system for blood levels of OSTN in humans is mandatory to address this question and is now ongoing in our laboratory. Although the bone–kidney association is important in renal pathophysiology, as represented by chronic kidney disease–mineral and bone disorder (CKD-MBD), we have not yet been able to evaluate Ostn expression in the bone. Clarification of the changes and regulation of Ostn expression in the bone during renal insufficiency awaits further investigations.

In the present study, we simulated therapeutic administration of OSTN in a clinical setting by overexpressing Ostn under the control of a liver-specific serum amyloid P promoter [25, 26]. OSTN acts in a way to enhance the NP actions locally by
antagonizing its clearance receptor NPR-C (Figure 7), which is different from direct administration of NPs. Therefore it could enhance local NP actions while minimizing its systemic effect, such as hypotension. In this regard, OSTN is somewhat similar to neprilysin inhibitors that enhance the local actions of NPs. The angiotensin receptor–neprilysin inhibitor (ARNI), which is now available in clinical settings, has been shown to exert potential cardio renal protective effects [19, 20, 44, 45]. Because OSTN can potentiate NP’s action, it is highly conceivable that OSTN may have similar benefits. Regarding the timing of OSTN administration, although pre-administration is possible in cases where the decreased renal blood flow is predictable, such as in transplantation and open-heart surgery, post-dosing will be the basis for most of AKI cases encountered in a clinical practice. Therefore the investigation of OSTN administration after developing insult is no doubt necessary as the next preclinical studies.

In conclusion, we have shown for the first time that Ostn overexpression can potentially ameliorate the progression of AKI to CKD. As exemplified by ARNI, an adequate potentiation of the local effects of NPs by OSTN may become a promising strategy for organ protection in various kidney diseases.

FIGURE 6: Effects of OSTN on the Wnt/β-catenin pathway activation evaluated by a luciferase assay and cGMP levels. (a) TopFlash reporter plasmid-transfected NRK52E cells were treated with TGFβ1 (10 ng/mL), ANP (10^{-8} mol/L), OSTN (10^{-7} mol/L) and 8-Br-cGMP (10^{-4} mol/L). The Wnt/β-catenin activity was detected by a luciferase assay. Bars show mean ± SEM. **P < 0.01, TGFβ1 versus TGFβ1 + ANP + OSTN; ††P < 0.01, TGFβ1 versus TGFβ1 + 8-Br-cGMP. n = 8–10. (b) cGMP levels of cell lysate treated by ANP and/or OSTN. Bars show mean ± SEM. **P < 0.01, vehicle versus ANP 10^{-9} + OSTN 10^{-7}. n = 3–4.

FIGURE 7: A schematic view of the proposed mechanism for the action of OSTN. OSTN can inhibit the degradation of ANP by antagonizing its clearance receptor NPR-C. ANP escaped from the degradation locally activates NPR-A, thereby increasing the intracellular levels of its second messenger cGMP. The Wnt/β-catenin pathway is inhibited via PKG.

SUPPLEMENTARY DATA
Supplementary data are available at ndt online.

ACKNOWLEDGEMENTS
We gratefully acknowledge Hikari Shibuta, Kazumi Saito and Naoko Hirano for technical assistance and Noriko Nakagawa and Miki Horikiri for secretarial assistance.

AUTHORS’ CONTRIBUTIONS
Y.N. and T.Kuwabara designed the study. N.M. and M.M. supervised the study. Y.N., D.F., Y.H., S.U., T.Kanki and R.D. performed the experiments. H.Y., K.P.M and T.H. measured the plasma OSTN levels. Y.N., T.Kuwabara and M.M. drafted the manuscript. T.Kuwabara, D.F., Y.H., K.P.M., Y.L., Y.K., N.M. and M.M. interpreted the results. H.W.-T. and Y.K. generated the OSTN knockout mice and A.Y. generated the liver-specific transgenic mice. All authors approved the final version of the manuscript.

FUNDING
This work was supported in part by research grants from JSPS KAKENHI (grants 23390225, 17K09706, 17K09704, 19K08728

Osteocrin’s renoprotective role in ischemia–reperfusion injury in mice
and 20K08611), the Integration Research for Agriculture and Interdisciplinary Fields from the National Agriculture and Food Research Organization and Japan Agency for Medical Research and Development (AMED; grants A26, JP18gm5010002, JP18gm0610011 and 16gm0610010h0004), Strategic Grant from the Center for Metabolic Regulation of Healthy Aging, Kumamoto University Faculty of Life Sciences (grants 009-0909005001011 and 204030114), Takeda Science Foundation (grant 09700220) and the Kidney Foundation, Japan (JKF19-21).

**DATA AVAILABILITY STATEMENT**

The data underlying this article are available in the text and its online supplementary material.

**CONFLICT OF INTEREST STATEMENT**

None declared.

**REFERENCES**

1. Belayev LY, Palevsky PM. The link between acute kidney injury and chronic kidney disease. *Curr Opin Nephrol Hypertens* 2014; 23: 149–154
2. Leung KC, Tonelli M, James MT. Chronic kidney disease following acute kidney injury-risk and outcomes. *Nat Rev Nephrol* 2013; 9: 77–85
3. Bonventre JV, Basile D, Liu KD et al. AKI: a path forward. *Clin J Am Soc Nephrol* 2013; 8: 1606–1608
4. Hoste EAJ, Kellum JA, Selby NM et al. Global epidemiology and outcomes of acute kidney injury. *Nat Rev Nephrol* 2018; 14: 607–625
5. Nakao K, Ogawa Y, Suga S et al. Molecular biology and biochemistry of the natriuretic peptide system. I: natriuretic peptides. *J Hypertens* 1992; 10: 907–912
6. Potter LR, Abbey-Hosch S, Dickey DM. Natriuretic peptides, their receptors, and cyclic guanosine monophosphate-dependent signaling functions. *Endocr Rev* 2006; 27: 47–72
7. Tamura N, Ogawa Y, Chusho H et al. Cardiac fibrosis in mice lacking brain natriuretic peptide. *Proc Natl Acad Sci* 2000; 97: 4239–4244
8. Kasahara M, Mukoyama M, Sugawara A et al. Ameliorated glomerular injury in mice overexpressing brain natriuretic peptide with renal ablation. *J Am Soc Nephrol* 2000; 11: 1691–1701
9. Suganami T, Mukoyama M, Sugawara A et al. Overexpression of brain natriuretic peptide in mice ameliorates immune-mediated renal injury. *J Am Soc Nephrol* 2001; 12: 2652–2663
10. Ogawa Y, Mukoyama M, Yokoi H et al. Natriuretic peptide receptor guanylyl cyclase-A protects podocytes from aldosterone-induced glomerular injury. *J Am Soc Nephrol* 2012; 23: 1198–1209
11. Lee CY, Huntley BK, McCormick DJ et al. Cenderitide: structural requirements for the creation of a novel dual particulate guanylyl cyclase receptor agonist with renal-enhancing in vivo and ex vivo actions. *Eur Heart J Cardiovasc Pharmacother* 2016; 2: 98–105
12. Kato Y, Mori K, Kasahara M et al. Natriuretic peptide receptor guanylyl cyclase-A pathway counters glomerular injury evoked by aldosterone through p38 mitogen-activated protein kinase inhibition. *Sci Rep* 2017; 7: 46624.
13. Sward K, Valsson F, Odencrants P et al. Recombinant human atrial natriuretic peptide in ischemic acute renal failure: a randomized placebo-controlled trial. *Crit Care Med* 2004; 32: 1310–1315
14. Sezai A, Hata M, Nino T et al. Results of low-dose human atrial natriuretic peptide infusion in nondialysis patients with chronic kidney disease undergoing coronary artery bypass grafting; the NU-HIT (Nihon University working group study of low-dose HANP infusion therapy during cardiac surgery) trial for CKD. *J Am Coll Cardiol* 2011; 58: 897–903
15. Lewis J, Salem MM, Chertow GM et al. Atrial natriuretic factor in oliguric acute renal failure. *Anatase Dis* 2000; 36: 767–774
16. Sackner-Bernstein JD, Skopicki HA, Aaronson KD. Risk of worsening renal function with nesiritide in patients with acutely decompensated heart failure. *Circulation* 2005; 111: 1487–1491
17. O’Connor CM, Starling RC, Hernandez AF et al. Effect of nesiritide in patients with acutely decompensated heart failure. *N Engl J Med* 2011; 365: 32–43
18. Riter HG, Redfield MM, Burnett JC et al. Nonhypotensive low-dose nesiritide has differential renal effects compared with standard-dose nesiritide in patients with acute decompensated heart failure and renal dysfunction. *J Am Coll Cardiol* 2006; 47: 2334–2335
19. Spannella F, Giuliatti F, Filipponi A et al. Effect of sacubitril/valsartan on renal function: a systematic review and meta-analysis of randomized controlled trials. *ESC Heart Failure* 2020; 7: 3487–3496
20. Solomon SD, McMurray JFV, Anand IS et al. Angiotensin- nephrilysin inhibition in heart failure with preserved ejection fraction. *N Engl J Med* 2019; 381: 1609–1620
21. Thomas G, Moffatt P, Saitou P et al. Osteocarin, a novel bone-specific secreted protein that modulates the osteoblast phenotype. *J Biol Chem* 2003; 278: 50653–50671
22. Nishizawa H, Matsuda M, Yamada Y et al. Muscling, a novel skeletal muscle-derived secretary factor. *J Biol Chem* 2004; 279: 19391–19395
23. Moffatt P, Thomas G, Sellin K et al. Osteocarin is a specific ligand of the natriuretic peptide clearance receptor that modulates bone growth. *J Biol Chem* 2007; 282: 36454–36462
24. Kita S, Nishizawa H, Okuno Y et al. Competitive binding of musclin to natriuretic peptide receptor 3 with atrial natriuretic peptide. *J Endocrinol* 2009; 201: 287–295
25. Kanai Y, Yasoda A, Mori KP et al. Circulating osteocarin stimulates bone growth by limiting C-type natriuretic peptide clearance. *J Clin Invest* 2017; 127: 4136–4147
26. Miyazaki T, Otani K, Chiba A et al. A new secretory peptide of natriuretic peptide family, osteocarin, suppresses the progression of congestive heart failure after myocardial infarction. *Circ Res* 2018; 122: 742–751
27. Gorski M, Tan A, Garnaa M et al. Genome-wide association study of kidney function decline in individuals of European descent. *Kidney Int* 2015; 87: 1017–1029
28. Nagai C, Minamino N. Direct chemiluminescence enzyme immunoassay for atrial natriuretic peptide in mammalian plasma using a PEGylated antibody. *Anal Biochem* 2014; 461: 10–16
29. Xiao L, Zhou D, Tan RJ et al. Sustained activation of Wnt/β-catenin signaling drives AKI to CKD progression. *J Am Soc Nephrol* 2016; 27: 1724–1740
30. Hafstatter T, Merryman WD, Gewin LS. Wnt/β-catenin in acute kidney injury and progression to chronic kidney disease. *Semin Nephrol* 2020; 40: 126–137
31. Coca SG, Singanamala S, Parikh CR. Chronic kidney disease after acute kidney injury: a systematic review and meta-analysis. *Kidney Int* 2012; 81: 442–448
32. Li N, Xi Y, Tinsley HN et al. Sulindac selectively inhibits colon tumor cell growth by activating the cGMP/PKG pathway to suppress Wnt/β-catenin signaling. *Mol Cancer Ther* 2013; 12: 1848–1859
33. Theilig F, Wu Q. ANP-induced signaling cascade and its implications in renal pathophysiology. *Am J Physiol Renal Physiol* 2015; 308: F1047–F1055
34. Burnett JC Jr, Opgenorth TJ, Granger JP. The renal action of atrial natriuretic peptide during control of glomerular filtration. *Kidney Int* 1986; 30: 16–19
35. Harris PJ, Skinner SL, Zhou J. The effects of atrial natriuretic peptide and glucagon on proximal glomerulo-tubular balance in anaesthetized rats. *J Physiol* 1988; 402: 29–42
36. Winaver J, Burnett JC, Tycz GM et al. ANP inhibits Na⁺-H⁺ antiport in proximal tubular brush border membrane: role of dopamine. *Kidney Int* 1990; 38: 1133–1140
37. Serafino A, Moroni N, Psaila R et al. Anti-proliferative effect of atrial natriuretic peptide on colorectal cancer cells: evidence for an Akt-mediated cross-talk between NHE-1 activity and Wnt/β-catenin signaling. *Biochim Biophys Acta* 2012; 1822: 1004–1018
38. Nakamura J, Sato Y, Kitay Y et al. Myofibroblasts acquire retinoic acid-producing ability during fibroblast-to-myofibroblast transition following kidney injury. *Kidney Int* 2019; 95: 526–539.
39. Watanabe-Takano H, Ochi H, Chiba A et al. Mechanical load regulates bone growth via periosteal osteocrin. *Cell Rep* 2021; 36: 109380.

40. Painter P, Roshanravan B. The association of physical activity and physical function with clinical outcomes in adults with chronic kidney disease. *Curr Opin Nephrol Hypertens* 2013; 22: 615–623

41. Kohzuki M, Kamimoto M, Wu XM et al. Renal protective effects of chronic exercise and antihypertensive therapy in hypertensive rats with chronic renal failure. *J Hypertens* 2001; 19: 1877–1882

42. Baria F, Kamimura MA, Aoike DT et al. Randomized controlled trial to evaluate the impact of aerobic exercise on visceral fat in overweight chronic kidney disease patients. *Nephrol Dial Transplant* 2014; 29: 857–864

43. Greenwood SA, Koufaki P, Mercer TH et al. Effect of exercise training on estimated GFR, vascular health, and cardiorespiratory fitness in patients with CKD: a pilot randomized controlled trial. *Am J Kidney Dis* 2015; 65: 425–434

44. McMurray JJ, Packer M, Desai AS et al. Angiotensin-neprilysin inhibition versus enalapril in heart failure. *N Engl J Med* 2014; 371: 993–1004

45. Packer M, Claggett B, Lefkowitz MP et al. Effect of neprilysin inhibition on renal function in patients with type 2 diabetes and chronic heart failure who are receiving target doses of inhibitors of the renin-angiotensin system: a secondary analysis of the PARADIGM-HF trial. *Lancet Diabetes Endocrinol* 2018; 6: 547–554

Received: 24.5.2021; Editorial decision: 23.9.2021