Canadian Science Publishing

ARTICLE

Inhibition of YAP activation attenuates renal injury and fibrosis in angiotensin II hypertensive mice

Junjie Zhang, Qian Xu, Fu Ren, Yueyang Liu, Ruiping Cai, Yang Yao, and Ming-Sheng Zhou

Abstract: The Hippo/YAP (yes-associated protein) pathway is an important signaling pathway to control organ development and tissue homeostasis. YAP is a downstream effector of the Hippo pathway and a critical mediator of mechanic stress. Hypertensive nephropathy is characterized with glomerular sclerosis stiffness and renal fibrosis. The present study investigated the role of YAP pathway in angiotensin (Ang) II hypertensive renal injury by using YAP activation inhibitor verteporfin. Ang II increased the protein expression of YAP in renal nucleus fraction, decreased phospho-YAP, and phospho-LATS1/2 (large tumor suppressors 1 and 2) expressions in renal cytoplasmic fraction, suggesting Ang II activation of renal YAP. Ang II significantly increased systolic blood pressure (SBP), proteinuria, glomerular sclerosis, and fibrosis; treatment with verteporfin attenuated Ang II–induced proteinuria and renal injury with a mild reduction in SBP. Moreover, Ang II increased the protein expressions of inflammatory factors including tumor necrosis factor α, interleukin 1β, and monocyte chemoattractant protein-1, and profibrotic factors including transforming growth factor β, phospho-Smad3 and fibronectin. Verteporfin reversed abovementioned Ang II–induced molecule expressions. Our results for the first time demonstrate that the activation of the YAP pathway promotes hypertensive renal inflammation and fibrosis, which may promote hypertensive renal injury. YAP may be a new target for prevention and treatment of hypertensive renal diseases.

Key words: angiotensin II, Hippo/YAP pathway, hypertensive nephropathy, inflammation, renal fibrosis.

Résumé : La voie de signalisation Hippo/YAP (pour « yes-associated protein ») joue un rôle important dans le contrôle du développement des organes et l'homéostasie des tissus. La YAP constitue un effecteur en aval de la voie de signalisation Hippo, ainsi qu'un médiateur essentiel du stress mécanique. La néphropathie hypertensive se caractérise par une sclérose rigide des glomérules et une fibrose rénale. La présente étude portait sur le rôle de la voie de signalisation YAP dans les lésions rénales hypertensives liées à l'angiotensine (Ang) II à l'aide de la vertéporfine, un inhibiteur de l'activation de la YAP. L'Ang II entraînait une augmentation de l'expression en protéines de la YAP dans la fraction nucléaire des reins, de même qu'une diminution de l'expression de p-YAP et de p-LATS1/2 dans la fraction cytoplasmique des reins, ce qui laissait entrevoir une activation de la YAP rénale par l'Ang II. L'Ang II entraînait une augmentation marquée de la tension artérielle systolique (TAS), une protéinurie, de la sclérose glomérulaire et de la fibrose, tandis que l'administration de vertéporfine permettait d'atténuer la protéinurie et les lésions rénales engendrées par l'Ang II, avec une légère diminution de la TAS. En outre, l'Ang II entraînait une augmentation de l'expression en protéines de facteurs inflammatoires, y compris le facteur de nécrose tumorale α, l'interleukine 1β et la MCP1 (pour « monocyte chemoattractant protein-1 »), ainsi que de facteurs profibrotiques, y compris le facteur de croissance transformant β, la phospho-Smad3 et la fibronectine. La vertéporfine permettait d'inverser l'expression des molécules ci-dessus que l'Ang II avait induite. Nos résultats sont les premiers à montrer que l'activation de la voie de signalisation YAP favorise l'inflammation et la fibrose rénales hypertensives, ce qui pourrait favoriser la survenue de lésions rénales hypertensives. La protéine YAP pourrait constituer une nouvelle cible dans la prévention et le traitement des maladies rénales hypertensives. [Traduit par la Rédaction]

Mots-clés : angiotensine II, voie de signalisation Hippo/YAP, néphropathie hypertensive, inflammation, fibrose rénale.

Introduction

Hypertensive nephropathy is an independent risk factor for the development of end-stage renal diseases. Chronic high blood pressure can damage endothelium and renal arteries to cause renal arterial thickness, glomerular sclerosis, and extracellular deposition and renal fibrosis (Seccia et al. 2017). Consequently, the renal tissue hardens and thickens, which is known as nephrosclerosis (Kotaro et al. 2015). Though the relationship between hypertension and chronic renal diseases is well established, the underlying mechanisms are still not completely understood.

The Hippo pathway is an evolutionarily conserved signaling pathway. Hippo signaling pathways consist of mammalian sterile 20-like kinases 1 and 2, salvador 1, large tumor suppressors 1 and 2 (LATS1/2), yes-associated protein (YAP)/TAZ (transcriptional coactivator with PDZ-binding motif), and other signal molecules (Hao et al. 2014). YAP is a transcription regulator and final effector of the Hippo pathway. In its phosphorylated form, YAP is

Received 16 September 2020. Accepted 1 March 2021.

J. Zhang,* Q. Xu,* R. Cai, Y. Yao, and M.-S. Zhou. Department of Physiology, Shenyang Medical College.
F. Ren. Department of Anatomy, Shenyang Medical College, Shenyang, China, 110034.
Y. Liu. Department of Pharmacology, Shenyang Pharmaceutical University, Shenyang, China.

Corresponding author: Ming-Sheng Zhou (email: zhoums1963@163.com).
*These authors equally contributed to this work.

sequestered in an inactive state. When the Hippo pathway is inhibited, dephosphorylated YAP is activated and translocated to the nucleus to interact with various transcription factors to regulate the transcription of target genes in which most of them are oncogenes that regulate cell proliferation, differentiation, tissue homeostasis, and organ size determination (Cordenonsi et al. 2014; Wang et al. 2018; Bonse et al. 2018). Therefore, the Hippo/YAP pathway is of great interest in tumor biology. Recent studies suggest that the Hippo/YAP pathway may play a role in the pathogenesis of some cardiovascular and renal diseases (Wang et al. 2018; Szeto et al. 2016).

Limited but compelling evidence suggest that the Hippo/YAP pathway has a significant role in the kidney and urinary tract development, podocyte homeostasis, and fibrotic and diabetic renal disease (Chen and Harris 2016). YAP subcellular location is sensitive to matric stiffness. On the stiff matrix, YAP translocates into the cell nuclei and is activated (Panciera 2017). Szeto et al. (2016) have shown that kidney stiffness after injury activates YAP pathway, which cooperates with the transforming growth factor (TGF) β/Smad signaling to induce renal fibrosis. Treatment with verteporfin, an inhibitor of YAP/TAZ with other transcription factors, attenuates renal fibrosis in the mice subjected to unilateral ureteral obstruction (Szeto et al. 2016). Increased expressions of YAP and its target genes have been reported in the diabetic kidney or human diabetic nephropathy (Ma et al. 2019; Chen and Harris 2016).

Hypertensive nephropathy is characterized with glomerular stiffness and nephrosclerosis. Ang II hypertensive nephropathy is associated with glomerular stiffness, renal fibrosis, and inflammation (Lei et al. 2018). Thus far, there is no literature showing that the Hippo/YAP pathway is activated in the hypertensive renal disease. Considering that the Hippo/YAP pathway is critical in sensing the renal mechanic stress and fibrosis, it is tempting to speculate that YAP activation plays an important role in the Ang II hypertensive nephropathy. In the present study, we used YAP activation inhibitor verteporfin to treat Ang II–hypertensive mice for 3 weeks. The results reported here demonstrate that YAP pathway has an important role in Ang II–induced renal injury.

Materials and methods

Animal and experimental protocols

Male 8-week-old C57BL/6 mice weighing 20–25 g were purchased from Vital River Laboratory Animal Technology Co., Ltd. (Beijing, China). All animal protocols comply with the international standards stated in the Guide for the Care and Use of Laboratory Animals and were approved by the Institutional Animal Care and Use Committee of Shenyang Medical College (Approved Animal Protocol #: 17-25). The mice were randomly divided into three groups and treated for 3 weeks: (*i*) Sham control (Ctr): Sham surgery with the implantation of an empty osmotic mini-pump (Alzet model 1002 D, DURECT Inco., Cupertino, CA, USA, $n = 6$); (*ii*) Ang II hypertensive group (Ang II): the implantation of an osmotic mini-pump plus Ang II infusion (1.1 mg/kg per day, Sigma–Aldrich, St. Louis, MO, USA, $n = 6$); (*iii*) Ang II with verteporfin treatment (Ang II/VE): the implantation of an osmotic mini-pump with Ang II plus verteporfin treatment (Selleckchem, Houston, TX, USA, $n = 6$). We have previously shown that Ang II infusion at this pressor dose for 2–3 weeks significantly induces hypertensive renal injury (Lei et al. 2018). Verteporfin was dissolved in 10% DMSO saline solution and administrated into the mice by intraperitoneal injection every other day at dosage of 60 mg/kg. The mice in the Ang II or the Ctr group were intraperitoneally injected with an equal volume of saline solution. Verteporfin is widely used as an inhibitor of YAP activation. The dose of verteporfin in the treatment of tumor or renal diseases in mice varies greatly from 5 to 100 mg/kg (i.p., every other day) (Szeto et al. 2016; Shin et al. 2020; Lui et al. 2019). Verteporfin inhibits YAP activation by interfering with the ability of YAP to interact with other proteins in the nucleus, therefore inhibiting YAP-dependent transcriptional gene expression (Gibault et al. 2016). In addition, verteporfin has been shown to inhibit translocation of YAP into the nuclei and decrease mRNA expression of YAP (Guillemette et al. 2017; Zhang et al. 2019). Systolic blood pressure (SBP) was measured by tail cuff method (Softron Biotechnology Inco., Beijing) on conscious mice. The mice were trained daily for 5 consecutive days of SBP measurement before the experiment started. SBP was measured at baseline (before the mini-pump implantation) and thereafter once a week. At least five successive readings were recorded and averaged as an individual blood pressure value for each mouse. At the end of the experiment, the urine was collected by squeezing the mice bladder to stimulate urination, and the urine was collected on a metal plate. The ratio of urine albumin/creatinine was determined by albumin-to-creatinine ration assay kit following by the manufacturer·s instructions (Nanjing Jiancheng Bioengineering Institute Co., Nanjing, China). The mice were euthanized by an overdose of anesthetic (5% chloral hydrate 0.4 mL/100 g i.p.). The kidneys were harvested then snap-frozen with liquid nitrogen.

Renal histological study

The renal tissues were processed for paraffin embedding and sectioned at 4 μm. Periodic acid–Schiff staining was performed to assess glomerular and tubular injury, including the glomerular sclerosis and the dilation of the mesangial matrix. The slides were photographed using a Leica fluorescence microscope. About 20 images per slide with at least one glomerulus per field were examined; a dark purple color in the glomeruli each field was recognized as sclerosis. The percentage of sclerotic area of glomeruli in each field was semi-quantitated using the Image Probe Plus version 6.0 image analysis system (Media Cybernetics, Bethesda, MD, USA). The percentage sclerotic area for the total of 20 images per slide was measured and averaged as one single value. Masson-Trichrome staining was used for evaluation of renal fibrosis. A semiquantitative analysis for the renal collagen content was performed by calculating percentage of positive stained areas in the total stained area using Image Pro Plus image analysis system (Media Cybernetics). The slide examination, image quantitation, and representative photomicrographs were taken in a blinded fashion without the knowledge of the experimental groups.

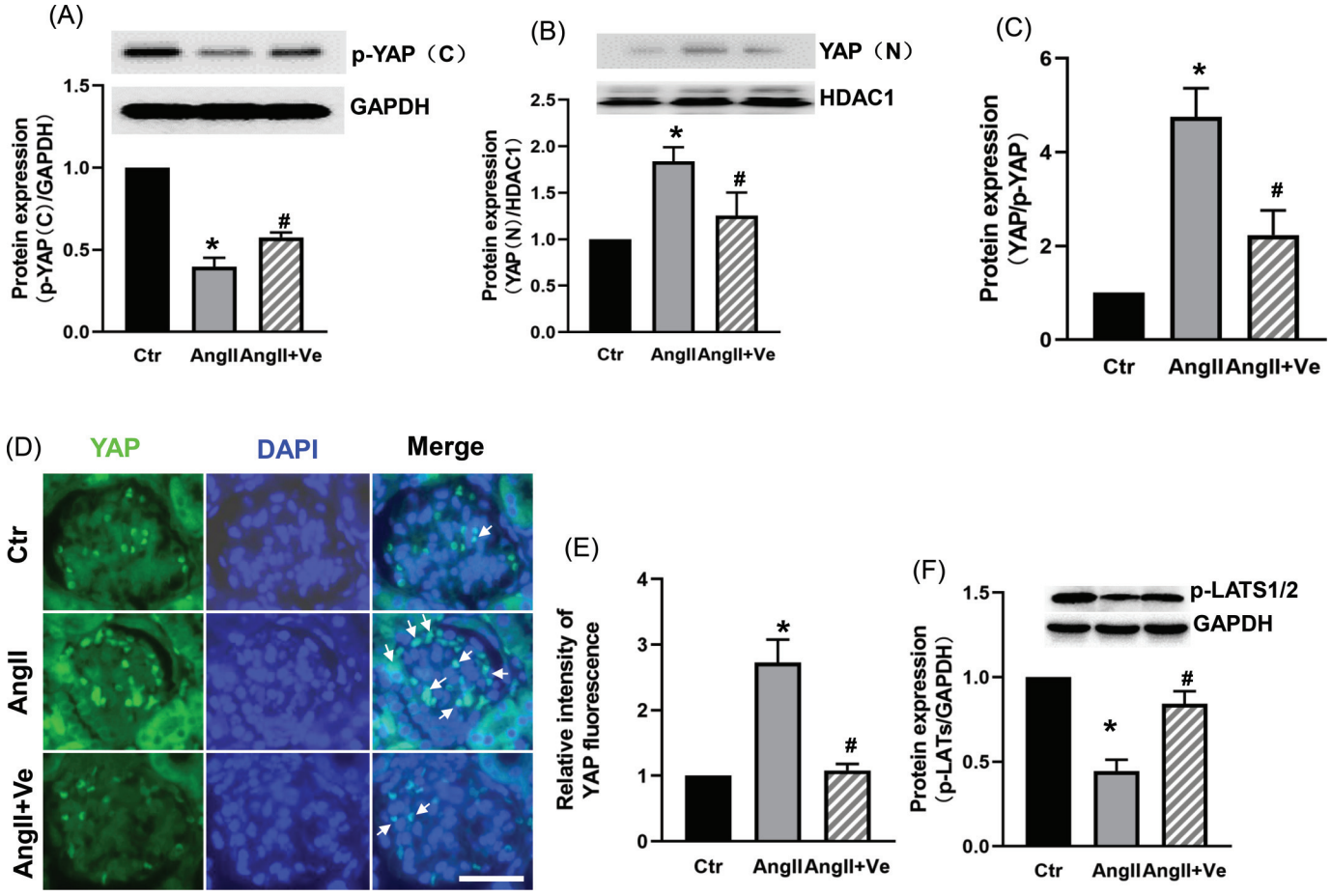
Immunofluorescence

After deparaffinization and hydration, renal sections were incubated with serum-free block solution to block nonspecific binding and microwaved for 30 min at 60 °C for antigen retrieval. The renal sections were then incubated with primary mouse anti-YAP (1:100 dilution, category (Cat) #: SC-101199, Santa Cruz Biotech Inc., Santa Cruz, CA) or rat anti-F4/80 antibodies (1:100 dilution, Cat #: 123101, Biolegend, San Diego, CA) overnight at 4 °C, followed by the incubation with fluorescein-conjugated goat anti-mouse secondary antibody for YAP (1:500 dilution) and donkey anti-rat second antibody for F4/80 (1:200 dilution, Beyotime Biotechnology, Shanghai, China) at 37 °C for 1 h. The nuclei were stained with DAPI. YAP fluorescence intensity and monocytes/macrophages (F4/80 positive cells) in the renal tissues were viewed using a fluorescence microscope. Twenty images in each section were examined and the number of positive cells (F4/80) per image was expressed by per mm^2 area of the image.

Western blot

The renal tissue was homogenized with the radioimmunoprecipitation assay buffer containing 1 mmol/L phenylmethylsulfonyl fluoride, 10 μg/mL aprotinin, and 10 μg/mL leupeptin. After the homogenization, the proteins in an aliquot of supernatant were quantified with Bio-Rad protein assay (Beyotime Biotechnology, Shanghai, China). Equal proteins (50 μg) were separated on SDS–PAGE and transferred to a polyvinylidene fluoride membrane. The membranes were incubated with primary antibodies against monocyte chemoattractant protein (MCP1, Cat#: SC-52701),

Fig. 1. Yes-associated protein (YAP) signaling was activated in the kidney of angiotensin (Ang) II–hypertensive mice. (A) The protein expression of phospho-YAP (p-YAP) in the renal cytosolic fraction; (B) the protein expression of YAP in the renal nucleus fraction; (C) the ratio of nucleus YAP/cytosolic p-YAP; (D) representative images of YAP immunofluorescence in renal section; (E) quantitative analysis of renal YAP fluorescence intensity; (F) the protein expression of p-LATS1/2 (large tumor suppressors 1 and 2) in the renal tissue. All data were expressed as mean ± standard error of the mean. $N = 6$; $^{*}p < 0.05$, vs. Ctr group; $\#p < 0.05$, vs. Ang II group; bar = 60 μm. Ctr: sham control group; Ang II: Ang II group; Ang II/VE: Ang II plus verteporfin treatment group. [Colour online.]



interleukin (IL) 1β (Cat #: SC-7884), tumor necrosis factor α (TNFα, Cat #: SC-52746), TGF-β, fibronectin (Cat #: SC-271098), connective tissue growth factor (CTGF, Cat #: SC-101586; Santa Cruz Biotechnology Inc.), phospho-Smad3 (p-Smad3, 1:1000, Cat #: 9520), p-LATS1/2 (1:1000 dilution, Cat #: 8654, Cell Signaling, Chicago, IL, USA), and GAPDH (Cat #: 60004, Proteintech Group, Danvers, MA, USA) at 4 °C overnight (all antibodies except the antibodies in Cell Signaling were in 1:500 dilution). Some renal tissues were used to separate cytosolic and nuclear fractions using NE-PER extraction reagent (Thermo Fisher Scientific, Rockford, IL) according to the manufacturer's protocol. YAP expression in the nuclear fraction and p-YAP expression in the cytoplasmic fraction of renal tissue were determined via incubation with primary antibodies against YAP (Cat #: SC-101199, Santa Cruz Biotechnology Inc.) and p-YAP (Cat #: 4911 S, Cell Signaling) for 1 h. The membranes were incubated with appropriate horseradish peroxidase–conjugated secondary antibodies for 1 h at room temperature. The specific bands of target proteins were detected by an Aplegen Omega Lum G Gel Documentation System (Aplegen Inc., Pleasanton, CA, USA) and quantified by National Institute of Health software Image J version 1.48. The membranes were reblotted with GADPH antibody (Santa Cruz Biotechnology Inc.) to serve as a loading control of cytosolic proteins and histone deacetylases (Cat #: SC-81598, Santa Cruz Biotechnology Inc.) to serve as a loading control of proteins of nuclear fraction.

Statistical analysis

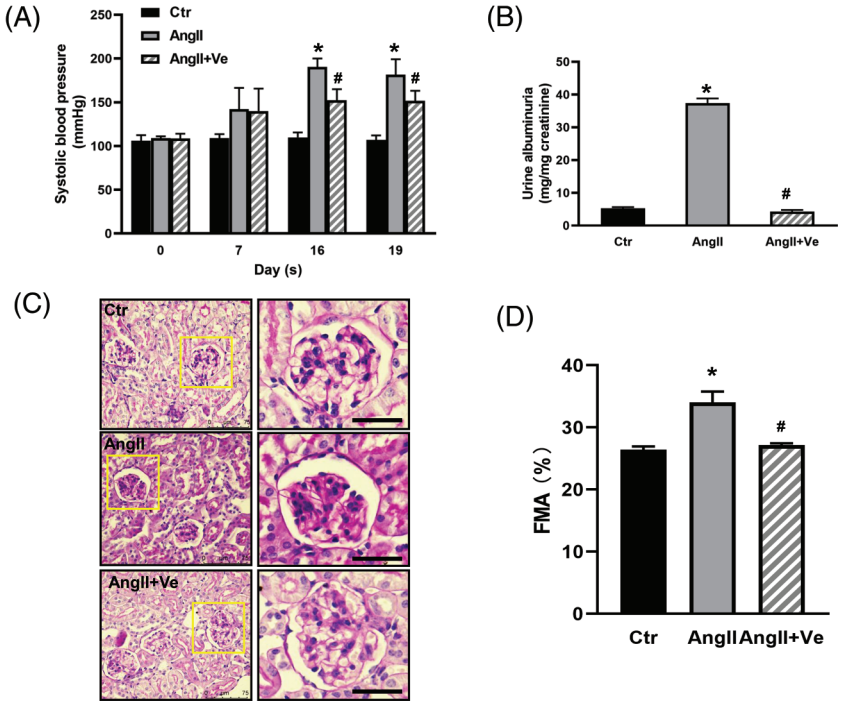
All data were expressed as mean ± standard error of the mean. Statistical analyses were performed using SPSS version 19.0 statistical software package (SPSS, Inc., Chicago, IL). Multiple group comparisons were analyzed by an analysis of variance (ANOVA) followed by Tukey's test. Statistical significance was considered when $p < 0.05$.

Results

The activation of YAP signaling in the kidney of Ang II–hypertensive mice

YAP is a key regulator and final effector of Hippo pathway. YAP activity is regulated by the Hippo pathway kinase LATS-dependent phosphorylation. Phosphorylated YAP is an inactive form in the cytosolic department. After dephosphorylation, YAP is activated and relocated into the nucleus (Dupont et al. 2011). To analyze YAP activation, the kidney tissues were separated into the nucleus and the cytoplasm fractions. Ang II decreased p-YAP in the cytoplasm (Fig. 1A) and increased the protein expression of YAP in the renal nucleus (Fig. 1B) and the ratio of nucleus YAP/cytosolic p-YAP (Fig. 1C). Treatment with verteporfin reversed Ang II–induced changes in YAP, p-YAP, and the ratio of cytosolic YAP/nucleus p-YAP (Figs. 1A–1C). Immunofluorescence showed that Ang II

Fig. 2. The inhibition of yes-associated protein (YAP) activation by verteporfin lowered (A) systolic blood pressure (SBP), (B) the ratio of urine protein/creatinine and (C, D) renal injury in angiotensin (Ang) II–hypertensive mice. (C) Representative images of renal section stained by periodic acid-Schiff (PAS) for evaluation of glomerular injury. (D) Quantitative analysis of glomerular sclerosis. $N = 6$; $^*p < 0.05$, vs. Ctr group; $\#p < 0.05$, vs. Ang II group; bar = 30 μm. Ctr: sham control group; Ang II: Ang II group; Ang II/VE: Ang II plus verteporfin treatment group. [Colour online.]



significantly increased total YAP fluorescence intensity and the colocalization of YAP fluorescence and nucleus staining DAPI (as indicated by arrow in Fig. 1D) in the glomerular area, which was reduced by verteporfin (Figs. 1D and 1E). The data indicates that YAP is activated in the kidney of Ang II–hypertensive mice, and verteporfin can effectively inhibit renal YAP activation. In addition, we determined p-LATS1/2 expression, an upstream molecule that regulates YAP activity. Ang II decreased p-LATS1/2 expression, and verteporfin increased LATS1/2 phosphorylation (Fig. 1F). These results suggest that Ang II induces YAP dephosphorylation and activation via downregulation of LATS1/2 phosphorylation.

Verteporfin reduced SBP, proteinuria, and renal injury

The infusion of Ang II for 3 weeks significantly increased SBP (185 ± 6 vs. 107 ± 5 mm Hg in the Ctr group, $p < 0.05$) and the ratio of urine protein/creatinine (37 ± 2.4 vs. 5.3 ± 0.7 μg/mg creatinine in the Ctr group, $p < 0.05$) in the mice. Treatment with verteporfin significantly reduced Ang II–induced increase in SBP (152 ± 6 vs. 185 ± 6 mm Hg in Ang II group, $p < 0.05$, Fig. 2A) and the ratio of urine protein/creatinine (4.3 ± 0.8 vs. 37 ± 2.4 μg/mg creatinine in Ang II group, $p < 0.05$, Fig. 2B). To assess whether the inhibition of YAP by verteporfin reduces renal injury in Ang II–hypertensive mice, the renal tissues were stained with periodic acid–Schiff. As shown in Figs. 2C and 2D, Ang II significantly increased glomerular sclerosis and glomerular matrix expansion, which were attenuated by verteporfin treatment.

Verteporfin inhibited renal fibrosis

Hypertensive nephropathy is associated with glomerular or tubular fibrosis. The activation of YAP pathway has been shown to promote renal fibrosis (Anorga et al. 2018; Xu et al. 2016). Consistent with our previous findings (Huang et al. 2018), Ang II significantly increased renal positive collagen-stained area, and treatment with verteporfin significantly reduced positive collagen-stained area (Figs. 3A and 3B). It has been shown that Ang II upregulates the expression of fibrotic factor TGF-β, which induces Smad phosphorylation and promotes Smad-dependent gene expression such as *fibronectin* and *CTGF*. We determined the expressions of TGF-β/Smad and their downstream molecules fibronectin and CTGF. The protein expressions of TGF-β, p-Smad 3, fibronectin, and CTGF were significantly increased in Ang II mice; treatment with verteporfin prevented significant increase of these protein expressions (Figs. 3C–3F). The data suggest that Ang II induces renal fibrosis via the activation of YAP-mediated TGF-β/Smad signaling.

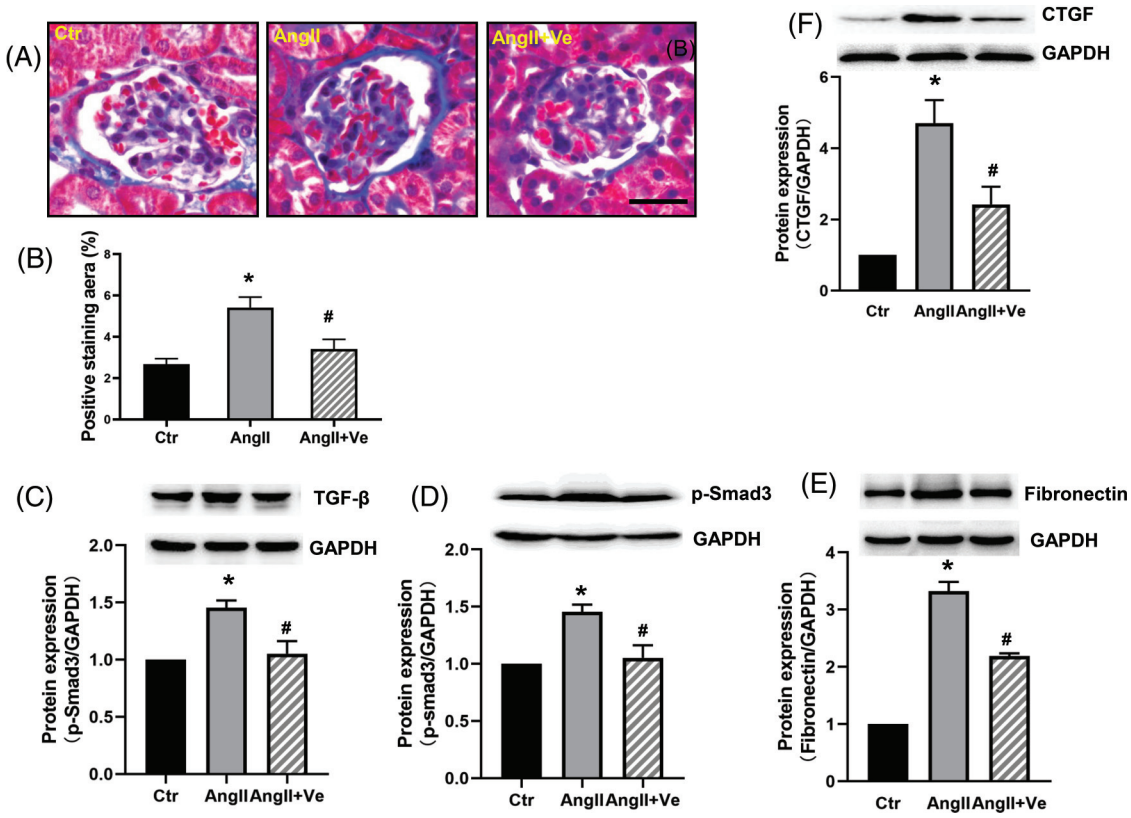
Verteporfin inhibited renal inflammation

Ang II hypertension is associated with renal inflammation. Recent studies have shown that the activated YAP pathway regulates macrophage polarization and inflammation (Zhou et al. 2019). We used immunofluorescence staining to determine monocyte/macrophage marker F4/80 expression in the renal tissue. As shown in Figs. 4A and 4B, F4/80 fluorescence intensity was increased in the glomerular area of Ang II–hypertensive mice, which was reduced in the Ang II/VE mice. Consistent with immunofluorescent findings, the protein expressions of proinflammatory cytokines TNFα, IL-1β, and MCP1 were increased in the kidney of Ang II mice, which were reduced in the Ang II/VE mice (Figs. 4C–4E).

Discussion

Hypertensive nephropathy manifests hypertensive nephrosclerosis and fibrosis (Seccia et al. 2017). Nephrosclerosis is a vital process that involves the changes in mesangial growth and death, podocyte apoptosis, and extracellular matrix (ECM) production and degradation (Wang et al. 2009). The Hippo/YAP pathway alters ECM production or degradation and the growth and death of glomerular endothelial cells and podocytes, which may contribute to various renal diseases (Cordenonsi et al. 2014; Noguchi et al. 2018; Rinschen

Fig. 3. (A and B) Verteporfin reduced renal fibrosis and (C) the expressions of transforming growth factor β (TGF-β), (D) phospho-Smad3 (p-Smad3), (E) fibronectin and (F) connective tissue growth factor (CTGF) in the angiotensin (Ang) II–hypertensive mice. (A) Representative images of renal section stained with Masson-Trichrome for the evaluation of renal fibrosis. (B) Quantitative analysis of positive collage-staining area in renal section. $N = 6$; $^{*}p < 0.05$, vs. Ctr group; $\#p < 0.05$, vs. Ang II group; bar = 30 μm. Ctr: sham control group; Ang II: Ang II group; Ang II/VE: Ang II plus verteporfin treatment group. [Colour online.]



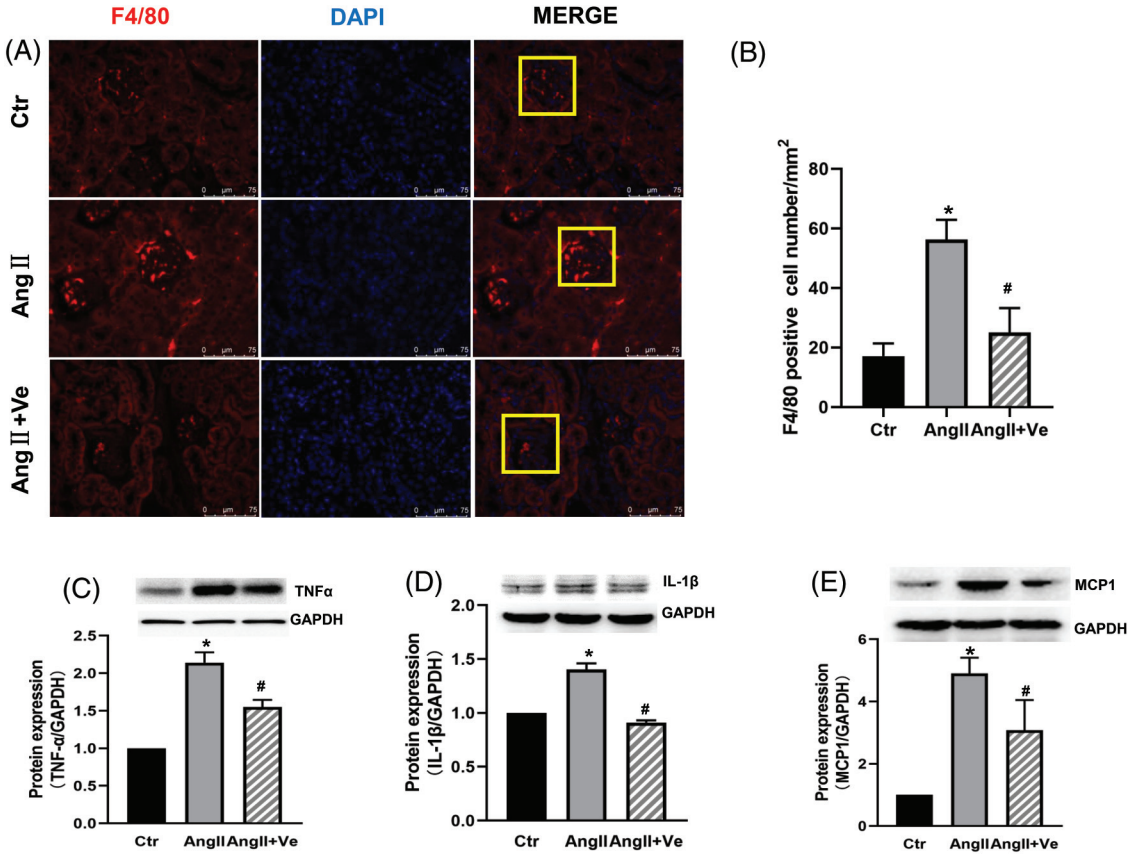
et al. 2017). In the present study, we demonstrate for first time that YAP expression is upregulated in the kidney of Ang II mice and that verteporfin reduces renal functional and structural damage, renal inflammation, and fibrosis associated with a mild reduction in blood pressure. These results suggest that the activation of YAP pathway plays an important role in hypertensive renal diseases.

The Hippo signaling pathway consists of a serine kinase cascade; LATS1/2 is a core upstream serine kinase of the Hippo signaling pathway, which negatively controls YAP activity by inducing YAP phosphorylation (Gill et al. 2018). Wennmann et al. (2014) shows that Ang II inactivates Hippo pathway via decreasing LATS kinase activity, which enhances nucleus shuttling of unphosphorylated YAP to activate YAP. Here we showed that Ang II increased renal nucleus YAP expression accompanied with decreased cytosolic p-YAP and p-LATS1/2. Therefore, Ang II may increase unphosphorylated YAP via the dephosphorylation of LATS1/2; unphosphorylated YAP then translocate into the nucleus to regulate YAP-dependent gene expression.

Ang II is a potent proinflammatory mediator. Ang II hypertensive nephropathy is often associated with chronic renal inflammation, which is characterized with increased immune cell infiltration and proinflammatory cytokine expression (Zhen et al. 2012; Wade et al. 2018). YAP signaling pathway has been shown to modulate tissue inflammation and reprograming macrophage polarization (Zhou et al. 2019). Specific deletion of YAP in the monocyte/macrophage lineage diminishes high-fat diet or lipopolysaccharide-induced hepatic inflammation and injury (Li et al. 2017; Li et al. 2018). YAP pathway increases monocyte-endothelial adhesion and the expression of vascular cell adhesion molecule 1 and intercellular adhesion molecule 1 in human umbilical vein endothelial cells; both of the molecules are critical for monocyte crossing of the vascular wall and migration into the tissue (Lv et al. 2018). We have recently shown that chemical depletion of macrophages by liposome-encapsulated clodronate effectively mitigates renal functional and structural injury and reduces renal expression of proinflammatory cytokines TNFα and IL-1β suggesting that macrophages play a critical role in the pathogenesis of Ang II–hypertensive renal diseases (Huang et al. 2018). In the present study, we showed that verteporfin inhibited renal infiltration of macrophage and reduced the expression of renal proinflammatory genes including *TNFα*, *MCP1*, and *IL-1β* in Ang II–hypertensive mice. Macrophages are main source of tissue TNFα and IL-1β, and TNFα has been implicated in the hypertensive renal diseases (Li et al. 2017). Therefore, we speculate that Ang II–induced renal inflammation may be at least in part mediated via YAP-dependent activation of macrophage. The hypothesis warrants to be further investigated.

Ang II hypertensive nephropathy is characterized with renal fibrosis and the deposition of ECM in the glomerulus. Ang II promotes renal fibrosis by upregulating TGF-β1/Smad signaling. TGF-β1 is a profibrotic regulator that stimulates the synthesis of matrix proteins and accumulation in the glomeruli (Liu et al. 2013). Renal matrix stiffness is also a factor in activating YAP/TAZ signaling (Szeto et al. 2016). It has been shown that the inhibition of YAP activation by verteporfin suppresses renal fibrosis in several animal models, such as diabetic nephropathy, polycystic kidney disease, and renal ureteral obstruction–induced renal fibrosis. The underlying mechanisms may involve the inhibition of YAP/

Fig. 4. Verteporfin attenuated angiotensin (Ang) II–induced renal inflammation and macrophage infiltration in hypertensive mice. (A) Representative images of immunofluorescence F4/80 in renal section; (B) Quantitative assessment of F4/80 expression. Red color indicates F4/80 and blue color indicates the nucleus. (C) The protein expression of tumor necrosis factor α (TNFα). (D) The protein expression of interleukin (IL) 1β. (E) The protein expression of monocyte chemoattractant protein (MCP) 1. $N = 6$; $^{*}p < 0.05$, vs. Ctr group; $\#p < 0.05$, vs. Ang II group; bar = 75 μm. Ctr: sham control group; Ang II: Ang II group; Ang II/VE: Ang II plus verteporfin treatment group. [Colour online.]



TAZ-mediated TGF-β1/Smad signaling (Rinschen et al. 2017; Liang et al. 2017). In Ang II–hypertensive mice, verteporfin reduces cardiac and vascular fibrosis (Lin et al. 2018). In addition, YAP can regulate the transcription and expressions of CTGF; CTGF and fibronectin are the downstream molecules of TGF-β/Smad signaling that participate in Ang II–induced fibrosis (Yang et al. 2018). The present study showed that verteporfin attenuated Ang II–induced renal fibrosis and reduced the expressions of TGF-β1, p-Smad3, fibronectin, and CTGF. These results indicate that the activation of YAP signaling contributes to Ang II–induced renal fibrosis in hypertensive mice.

It should be noted that in the present study the inhibition of YAP by verteporfin resulted in a mild but significant reduction in SBP. The hemodynamic plays an important role in hypertensive renal injury. Ang II–induced renal injury is blood pressure–dependent and blood pressure–independent, so our study cannot exclude the possibility that reduction in blood pressure per se may partially contribute to verteporfin improvement of renal injury; however, the mice in Ang II/VE group are still hypertensive, with a small reduction in SBP, and treatment with verteporfin resulted in obvious improvement of renal injury. Therefore, we surmise that renal beneficial effects of verteporfin is mainly mediated by inhibition of YAP activation. Next, the present study did not test verteporfin effects in normal mice. The results from vertorporfin-treated normal mice may help us in understanding verteporfin's effects in normal mice. Even so, it should not affect our conclusion that the activation of YAP pathway, at least in part, contributes to Ang II–induced renal injury.

In conclusion, the present study demonstrates that the YAP pathway plays a pivotal role in Ang II–hypertensive renal injury and the inhibition of YAP activation by verteporfin reduces renal inflammation and fibrosis, therefore mitigating hypertensive renal injury. The present study supports the notion that YAP may be a novel target for the prevention and treatment of hypertensive renal disease, in particular some renin-dependent hypertension.

Conflict of interest statement

All authors declare no conflicts of interest.

Author contributions

JZ: Contributed to acquisition of major experimental data and statistical analysis. QX: Contributed to acquisition of experimental data, statistical analysis and helped to write the draft of the manuscript. FR: Contributed to acquisition of data. YL: Contributed to acquisition of experimental data. LC: Contributed to acquisition of experimental data. YY: Contributed to acquisition of experimental data. MSZ: Contributed to the conception and design of the work, and writing the draft of the manuscript.

Data availability

The data used to support the findings of this study are included in the article.

Acknowledgement

This work was supported by the grants from the National Natural Science Foundation of China (Nos. 81670384, 81970357), Research Fund of Shenyang Medical College (20191031) to MSZ. Science and Technology Planning Project of Liaoning Province (2019-ZD-0337) and Shenyang Medical College Clinical Research Development Fund (No. 20187083) to QX.

References

Anorga, S., Overstre, J.M., Falke, L.L., Tang, J., Goldschmeding, R.G., Higgins, P.J., et al. 2018. Deregulation of Hippo-TAZ pathway during renal injury confers a fibrotic maladaptive phenotype. FASEB J. **32**(5): 2644–2657. doi:10.1096/fj.201700722R. PMID:29298862.

Bonse, J., Wennmann, D.O., Kremerskothen, J., Weide, T., Michgehl, U., Pavenstädt, H., and Vollenbröker, B. 2018. Nuclear YAP localization as a key regulator of podocyte function. Cell death Dis. **9**(9): 850. doi:10.1038/s41419-018-0878-1. PMID:30154411.

Chen, J., and Harris, R.C. 2016. Interaction of the EGF receptor and the hippo pathway in the diabetic kidney. J. Am. Soc. Nephrol. **27**(6): 1689–1700. doi:10.1681/ASN.2015040415. PMID:26453611.

Cordenonsi, M., Dupont, S., and Piccolo, S. 2014. The biology of YAP/TAZ: hippo signaling and beyond. Physiol. Rev. **94**(4): 1287–1312. doi:10.1152/physrev.00005.2014. PMID:25287865.

Dupont, S., Morsut, L., Aragona, M., Enzo, E., Giulitti, S., Cordenonsi, M., et al. 2011. Role of YAP/TAZ in mechanotransduction. Nature, **474**(7350): 179–183. doi:10.1038/nature10137. PMID:21654799.

Gibault, F., Corvaisier, M., Bailly, F., Hu, G., Melnyk, P., and Cotelle, P. 2016. Non-photoinduced biological properties of verteporfin. Curr. Med. Chem. **23**(11): 1171–1184. doi:10.2174/0929867323666160316125048. PMID:26980565.

Gill, M.K., Christova, T., Zhang, Y.Y., Gregorieff, A., Zhang, L., Narimatsu, M., et al. 2018. A feed forward loop enforces YAP/TAZ signaling during tumorigenesis. Nat. Commun. **9**(1): 3510. doi:10.1038/s41467-018-05939-2. PMID:30158528.

Guillemette, S., Rico, C., Godin, P., Boerboom, D., and Paqu, M. 2017. In vitro validation of the hippo pathway as a pharmacological target for canine mammary gland tumors. J. Mammary Gland Biol. Neoplasia, **22**(3): 203–214. doi:10.1007/s10911-017-9384-9. PMID:28822004.

Hao, J., Zhang, Y., Wang, Y., Ye, R., Qiu, J., Zhao, Z., and Li, J. 2014. Role of extracellular matrix and YAP/TAZ in cell fate determination. Cell. Signal. **26**(2): 186–191. doi:10.1016/j.cellsig.2013.11.006. PMID:24216612.

Huang, L., Wang, A., Hao, Y., Li, W., Liu, C., Yang, Z., et al. 2018. Macrophage depletion lowered blood pressure and attenuated hypertensive renal injury and fibrosis. Front. Physiol. **9**: 473. doi:10.3389/fphys.2018.00473. PMID:29867533.

Kotaro, H., Nobuo, T., Go, K., Kentaro, K., Masahiro, S., Akihiro, S., et al. 2015. Glomerular density in biopsy-proven hypertensive nephrosclerosis. Am. J. Hypertens. **28**(9): 1164–1171. doi:10.1093/ajh/hpu267. PMID:25631380.

Lei, H., Wang, A., Yun, H., Li, W., Chang, L., Yang, Z., et al. 2018. Macrophage depletion lowered blood pressure and attenuated hypertensive renal injury and fibrosis. Front. Physiol. **9**: 473. doi:10.3389/fphys.2018.00473. PMID:29867533.

Li, L., Zhou, J., Li, Q., Xu, J., Qi, J., and Bian, H. 2018. The inhibition of Hippo/Yap signaling pathway is required for magnesium isoglycyrrhizinate to ameliorate hepatic stellate cell inflammation and activation. Biomed. Pharmacother. **106**: 83–91. doi:10.1016/j.biopha.2018.06.102. PMID:29957470.

Li, X., Lin, Y., Zhou, H., Li, Y., Wang, A., Wang, H., and Zhou, M.S. 2017. Puerarin protects against endothelial dysfunction and end-organ damage in Ang II-induced hypertension. Clin. Exp. Hypertens. **39**(1): 58–64. doi:10.1080/10641963.2016.1200603. PMID:28060542.

Liang, M., Yu, M., Xia, R., Song, K., Wang, J., Luo, J., et al. 2017. Yap/taz deletion in Gli+ cell-derived myofibroblasts attenuates fibrosis. J. Am. Soc. Nephrol. **28**(11): 3278–3290. doi:10.1681/ASN.2015121354. PMID:28768710.

Lin, M., Yuan, W., Su, Z., Lin, C., Huang, T., Chen, Y., and Wang, J. 2018. Yes-associated protein mediates angiotensin II-induced vascular smooth muscle cell phenotypic modulation and hypertensive vascular remodelling. Cell Prolif. **51**(6): e12517. doi:10.1111/cpr.12517. PMID:30156340.

Liu, G., Li, Y., Huang, X., Wei, L., Chen, H.Y., Shi, Y., et al. 2013. Disruption of Smad7 promotes ANG II-mediated renal inflammation and fibrosis via Sp1-TGF-β/Smad3-NF.κB-dependent mechanisms in mice. PLoS ONE, **8**(1): e53573. doi:10.1371/journal.pone.0053573. PMID:23301086.

Lui, J.W., Xiao, S., Ogomori, K., Hammarstedt, J.E., Little, E.C., and Lang, D. 2019. The efficiency of verteporfin as a therapeutic option in pre-clinical models of melanoma. J. Cancer, **10**(1): 1–10. doi:10.7150/jca.27472. PMID:30662519.

Lv, Y., Kim, K., Sheng, Y., Cho, J., Qian, Z., Zhao, Y.Y., et al. 2018. YAP controls endothelial activation and vascular inflammation through TRAF6. Circ. Res. **123**(1): 43–56. doi:10.1161/CIRCRESAHA.118.313143. PMID:29794022.

Ma, R., Ren, J.M., Li, P., Zhou, Y.J., Zhou, M.K., Hu, Z., and Xiao, X.Y. 2019. Activated YAP causes renal damage of type 2 diabetic nephropathy. Eur. Rev. Med. Pharmacol. Sci. **23**(2): 755–763. doi:10.26355/eurrev_201901_16890. PMID:30720184.

Noguchi, S., Saito, A., and Nagase, T. 2018. YAP/TAZ signaling as a molecular link between fibrosis and cancer. Int. J. Mol. Sci. **19**(11): 3674. doi:10.3390/ijms19113674. PMID:30463366.

Panciera, T., Azzolin, L., Cordenonsi, M., and Piccolo, S. 2017. Mechanobiology of YAP and TAZ in physiology and disease. Nat. Rev. Mol. Cell. Biol. **18**(12): 758–770. doi:10.1038/nrm.2017.87. PMID:28951564.

Rinschen, M.M., Grahammer, F., Hoppe, A.-K., Kohli, P., Hagmann, H., Kretz, O., et al. 2017. YAP-mediated mechanotransduction determines the podocyte's response to damage. Sci. Signal. **10**(474): eaaf8165. doi:10.1126/scisignal.aaf8165. PMID:28400537.

Seccia, T.M., Caroccia, B., and Calo, L.A. 2017. Hypertensive nephropathy. Moving from classic to emerging pathogenetic mechanisms. J. Hypertens. **35**(2): 205–212. doi:10.1097/HJH.0000000000001170. PMID:27782909.

Shin, S.M., Kim, J.S., Park, S.W., Jun, S.Y., Kweon, H.J., Choi, D.K., et al. 2020. Direct targeting of oncogenic RAS mutants with a tumor-specific cytosol-penetrating antibody inhibits RAS mutant-driven tumor growth. Sci. Adv. **6**(3): eaay2174. doi:10.1126/sciadv.aay2174. PMID:31998840.

Szeto, S.G., Narimatsu, M., Lu, M., He, X., Sidiqi, A.M., Tolosa, M.F., et al. 2016. YAP/TAZ Are Mechanoregulators of TGF-beta-Smad Signaling and Renal Fibrogenesis. J. Am. Soc. Nephrol. **27**(10): 3117–3128. doi:10.1681/ASN.2015050499. PMID:26961347.

Wade, B., Petrova, G., and Mattson, D.L. 2018. Role of immune factors in angiotensin II-induced hypertension and renal damage in Dahl salt-sensitive rats. Am. J. Physiol. Regul. Integr. Comp. Physiol. **314**(3): R323–R333. doi:10.1152/ajpregu.00044.2017. PMID:29118017.

Wang, G., Lai, F.M.-M., Kwan, B.C.-H., Lai, K.-B., Chow, K.-M., Li, P.K.-T., and Szeto, C.-C. 2009. Podocyte Loss in Human Hypertensive Nephrosclerosis. Am. J. Hypertens. **22**(3): 300–306. doi:10.1038/ajh.2008.360. PMID:19131934.

Wang, J., Liu, S., Heallen, T., and Martin, J. 2018. The Hippo pathway in the heart: pivotal roles in development, disease, and regeneration. Nat. Rev. Cardiol. **15**(11): 672–668. doi:10.1038/s41569-018-0063-3. PMID:30111784.

Wennmann, D.O., Vollenbröker, B., Eckart, A.K., Bonse, J., Erdmann, F., Wolters, D.A., et al. 2014. The Hippo pathway is controlled by Angiotensin II signaling and its reactivation induces apoptosis in podocytes. Cell Death Dis. **5**(11): e1519–e1519. doi:10.1038/cddis.2014.476. PMID:25393475.

Xu, J., Li, P.X., Wu, J., Gao, Y.J., Yin, M.X., Lin, Y., et al. 2016. Involvement of the Hippo pathway in regeneration and fibrogenesis after ischaemic acute kidney injury: YAP is the key effector. Clin. Sci. **130**(5): 349–363. doi:10.1042/CS20150385. PMID:26574480.

Yang, W., Han, W., Qin, A., Wang, Z., Xu, J., and Qian, Y. 2018. The emerging role of Hippo signaling pathway in regulating osteoclast formation. J. Cell. Physiol. **233**(6): 4606–4617. doi:10.1002/jcp.26372. PMID:29219182.

Zhang, L., He, X., Liu, X., Zhang, F., Huang, L.F., Potter, A.S., et al. 2019. Single-cell transcriptomics in medulloblastoma reveals tumor-initiating progenitors and oncogenic cascades during tumorigenesis and relapse. Cancer Cell, **36**(3): 302–318e.307. doi:10.1016/j.ccell.2019.07.009. PMID:31474569.

Zhen, L., Xiao, R.H., and Hui, Y.L. 2012. Smad3 mediates ANG II-induced hypertensive kidney disease in mice. Am. J. Physiol. Renal. Physiol. **302**(8): F986–F997. doi:10.1152/ajprenal.00595.2011. PMID:22237801.

Zhou, X., Li, W., Wang, S., Zhang, P., Wang, Q., Xiao, J., et al. 2019. YAP aggravates inflammatory bowel disease by regulating M1/M2 macrophage polarization and gut microbial homeostasis. Cell Rep. **27**(4): 1176–1189. e1175. doi:10.1016/j.celrep.2019.03.028. PMID:31018132.