ORIGINAL PAPER

Capillary rarefaction and altered renal development: the imbalance between pro- and anti-angiogenic factors in response to angiotensin II inhibition in the developing rat kidney

Kee Hwan Yoo[1] · Hyung Eun Yim[1,2] · Eun Soo Bae[1] · Young Sook Hong[1]

Abstract

Proper and timely assembly of the kidney vasculature with their respective nephrons is crucial during normal kidney development. In this study, we investigated the effects of enalapril (angiotensin-converting enzyme inhibitor) on angiogenesis-related gene expression and microvascular endothelium related to glomelular and tubular changes in the neonatal rat kidney. Enalapril-treated rats had higher tubular injury scores and lower glomerular maturity grades than those of untreated rats. In the enalapril-treated group, intrarenal angiopoietin-2, Tie-2, and thrombospondin-1 protein expression increased, whereas intrarenal angiopoietin-1 protein expression decreased. JG12-positive glomerular and peritubular capillary staining was reduced in the enalapril-treated rat kidney. The number of JG12-positive capillary endothelial cells was directly correlated with glomerular maturation grade and was inversely related with the tubular injury. Our findings suggest the imbalance between pro- and anti-angiogenic factors may be implicated in the loss of capillaries in associated with impaired nephrogenesis after angiotensin II blockade in the developing rat kidney.

Keywords Angiopoietins · Neovascularization · Pathologic · Renin-angiotensin system · Thrombospondin-1

Introduction

The establishment of proper nephrovascular units is essential for the development of a functioning kidney necessary for independent extrauterine life (Gomez et al. 1997; Sequeira-Lopez and Gomez 2011). Some structural challenges are required as the kidney becomes vascularized during embryogenesis, including the formation of the renal arterial tree and glomerular capillaries and the alignment of the vasa recta and peritubular capillaries (Herzlinger and Hurtado 2014). Formation of glomerular capillaries is regulated by the balance between pro- and anti-angiogenic factors universal to other blood vessels (Abrahamson 2009; Tufro-McReddie et al. 1997). The arrangement of the post-glomerular capillary beds with the renal tubules is dependent on several angiogenic signaling pathways controlled by the renal tubules (Madsen et al. 2010). Maintenance of the glomerular capillaries help reserve glomerular filtration rate and preservation of the peritubular capillaries in the interstitium is vital for supplying oxygen and nutrition to tubular and interstitial cells (Herzlinger and Hurtado 2014).

Renal vascular homeostasis is tightly regulated by the balance between pro- and anti-angiogenic factors in healthy kidneys. However, this balance is disrupted in chronic kidney disease, resulting in an anti-angiogenic environment that induces loss of the peritubular capillaries (Kida et al. 2014). Angiopoietins (Angs), a family of vascular growth factors, play a significant role in the development of the kidney vasculature (Maisonpierre et al. 1997; Woolf et al. 2009). Ang-1, Ang-2, and the receptor tyrosine kinase Tie-2 are highly expressed in developing kidneys (Kolatsi-Joannou et al. 2001). Binding of Ang-1 to the Tie-2 receptor supports pericyte attachment and vessel maturation. Ang-2 antagonizes Ang-1 and prevents Tie-2 activation by competitively binding Tie-2. Ang-1 exerts anti-inflammatory and pro-angiogenic activities, whereas Ang-2 has opposite effects (Augustin et al. 2009; Maisonpierre et al. 1997).

✉ Hyung Eun Yim
he-yim@hanmail.net

1 Department of Pediatrics, College of Medicine, Korea University, Seoul, South Korea

2 Department of Pediatrics, Korea University Ansan Hospital, 123, Jeokgeum-ro, Danwon-gu, Ansan-si, Gyeonggi-do 15355, South Korea

Angs are implicated in renovascular maturation in parallel with VEGF-A, which has recognized roles in metanephric endothelial differentiation and survival (Eremina et al. 2003; Gnudi et al. 2015). Thrombospondins (TSPs) comprise a family of extracellular matrix proteins that control tissue genesis and remodeling. TSP-1 plays a role as a natural inhibitor of angiogenesis and directly initiates endothelial cell apoptosis (Guo et al. 1997; Zhang and Lawler 2007). TSP-1 expression has been shown to be positively associated with the loss of glomerular and peritubular capillaries (Bige et al. 2012).

All components of the renin-angiotensin system (RAS) are exhibited temporospatially in the developing kidney (Sequeira-Lopez et al. 2015). Blockade of the RAS with angiotensin converting enzyme (ACE) inhibitors in neonatal rats induces consistently reproducible renal abnormalities that persist after treatment, indicating an important role for the RAS in nephrovascular development (Friberg et al. 1994). A representative study by Tufro-McReddie et al. (1995) reveals impaired angiotensin II signaling leads to reduced glomerular size, tubular atrophy, and pathological changes in pre-glomerular circulation. A more recent study has shown that the growth actions of angiotensin II are not restricted to the renal arterioles and extend to the post-glomerular vasculature (Madsen et al. 2010). However, the actual events and molecules that control the development of the kidney microvasculature by the RAS remain unclear.

Since rats are born with immature kidneys, the neonatal rat model appears suitable for studying the mechanisms of renal development in the human fetus (de Almeida et al. 2017). Nephrogenesis is achieved prenatally in the human, but it continues until postnatal day 10 in the rat (Gomez et al. 1997; Hartman et al. 2007). We reported previously that inhibiting the ACE for the first 7 days after birth induced improper VEGF-A/VEGF receptor signaling in newborn rat kidneys. Expression of pro-angiogenic platelet-derived growth factor (PDGF)-B and its receptor-β were not changed after ACE inhibition (Yim et al. 2016). In the present study, we hypothesized that additional pro- and anti-angiogenic players contribute to the altered angiogenic response after the blockade of the RAS in developing kidneys. We investigated whether the altered intrarenal activities of Ang-1, Ang-2, Tie-2, and TSP-1 are involved in renal microvascular abnormalities following neonatal ACE inhibition. We quantified the loss of capillary density using aminopeptidase P (JG12, a specific endothelial marker) in newborn rat kidneys exposed to the RAS inhibitor. The associations between capillary density and glomerular and tubular changes were also assessed in our experimental model.

Materials and methods

Ethical approval

Our experimental protocol was approved by the Animal Experimentation Ethics Committee of Korea University and was carried out under the guidelines of the National Institutes of Health Guide for the Care and Use of Laboratory Animals. The authors have taken all steps to minimize the animals' pain and suffering.

Animal preparation

Neonatal rat pups from three pregnant Sprague-Dawley rats were breastfed by their mother throughout the study. Both male and female pups were used. Enalapril (Sigma Chemical Co., St. Louis, MO, USA) (30 mg/kg, n = 15) or vehicle (control group, n = 12) was given to newborn rats daily via an orogastric tube beginning at birth. This dose of enalapril is known to effectively block the effects of angiotensin II (Gomez et al. 1988). The rats were killed at 8 days of age (50 mg/kg i.p. pentobarbital sodium), and their kidneys were harvested and processed. The right kidney from each rat was used for light microscopy and immunohistochemistry, and the left kidney was used for Western blot analysis.

Histological examination

Hematoxylin and eosin staining was carried out as described previously to detect renal histological changes (Yim et al. 2009). Tubular injury grade was scored semiquantitatively in a blinded manner in at least 20 randomly chosen grid fields of the cortex ($25 \times 25\ \mu m^2$ cortex) per kidney section (400× magnification) from each of ten rats as previously described (Chen et al. 2013; Takaori et al. 2016). Tubular injury was defined as tubular dilation, tubular atrophy, tubular cast formation, sloughing of tubular epithelial cells, and vacuolization of the epithelium. The tubules were evaluated according to the following scoring system: 0 = no tubular injury; 1, < 25% tubules injured; 2, up to 50% tubules injured; 3, up to 75% tubules injured; and 4, > 75% tubules injured. The maturation stage of the glomeruli within each field of view was classified from 0 to 3 using an adaptation of a method published previously (Sutherland et al. 2011). Glomeruli were graded as stage 0 if they were in the early stages of development (vesicle, comma-shape, S-shape, or capillary loop stages). Stage 1 presented immature fully shaped glomeruli with at least half of the glomerular tuft lined with darkly stained cells and a densely cellular glomerular tuft. Stage 2 glomeruli comprised less than half of the glomerular circumference lined with dark-staining epithelial cells, and

stage 3 glomeruli (most mature) showed no darkly stained layer of cells surrounding the glomerular tuft and a more open glomerular tuft. Glomerular maturity was determined by grading at least 20 glomeruli per section area and calculating the mean from each of ten rats. The glomeruli in the cortex were identified at 400× magnification using a double blind method. Counts were performed randomly throughout all fields.

Western blotting

Equal amounts of protein (5–15 μg) were subjected to 10% SDS-PAGE, and the separated proteins were transferred to nitrocellulose membranes (KPL, Gaithersburg, MD). The membranes were blocked in 5% skim milk with TBS-T [0.05% Tween 20 in 50 mM of Tris, 150 mM of NaCl, and 0.05% NaN_3 (pH 7.4)] at room temperature for 1 h. The membranes were washed twice in TBS-T and incubated for 18 h at 4 °C with primary antibodies against Ang-1 (dilution 1:500), Ang-2 (1:200), Tie-2 (1:500), and TSP-1 (1:400). All primary antibodies were purchased from Santa Cruz Biotechnology (Santa Cruz, CA). The membranes were washed twice with TBS-T and incubated for 60 min with anti-rabbit IgG (Ap132p, 1:1000; Millipore, Temecula, CA), anti-mouse IgG (474-1806; Kirkegaard & Perry Laboratories, Inc., Gaithersburg, MD), and anti-goat IgG (HAF-109; R&D Systems, Minneapolis, MN) at room temperature. To control for equal loading, ß-actin (1:1000; Cell Signaling Technology, Danvers, MA) and anti-mouse IgG conjugated horseradish peroxidase (1:1000; Cell Signaling Technology, Danvers, MA) were used as primary and secondary antibodies using the same method described above. X-rays were scanned using the Epson GT-9500 (Seiko Corp, Nagano, Japan), and the results were quantified by a computerized densitometer (Image PC Alpha 9; National Institutes of Health, Bethesda, MD). The relative expression ratio of each protein band normalized to the each loading reference control (ß-actin) was compared between the two groups.

Immunohistochemistry

Ten to twelve kidneys in each group were selected for representative immunohistochemistry of Ang-1, Ang-2, Tie-2, and TSP-1 using an avidin–biotin immunoperoxidase method (Vectastain ABC kit, Burlingame, CA). Immunohistochemistry was performed as described previously (Yim et al. 2009). Primary antibodies against Ang-1 (1:500), Ang-2 (1:200), Tie-2 (1:500), and TSP-1 (1:400) were used. All the primary antibodies were purchased from Santa Cruz Biotechnology (Santa Cruz, CA). The primary antibody was substituted with phosphate buffered saline as a negative control. The secondary antibodies were peroxidase-conjugated anti-rabbit IgG antibodies (1:200; Millipore, Temecula). Images were captured

using an Olympus microscope (Tokyo, Japan). All images were viewed using Olympus CH20i Trinocular microscope and captured using an Olympus DP73 camera (Olympus, Tokyo, Japan).

Capillary density

Glomerular and peritubular capillary densities were assessed after immunostaining for JG12 using a point detection method. JG12 is a novel discriminatory marker of blood vessel endothelial cells (Sun et al. 2012). A mouse JG12 monoclonal antibody (1:50; Santa Cruz Biotechnology, Santa Cruz, CA) was used for immunohistochemistry performed using a DAB Kit according to the manufacturer's instructions. Capillary density was measured by counting the numbers of squares in 10×10 grids at 400× magnification that contained JG-12-positive capillary staining in the renal cortex, and mean JG12 staining in 20 randomly selected grid fields of the cortex per kidney section from ten rats was determined in a blinded manner. The relationships between capillary density and glomerular maturity or tubulointerstitial injury were also determined in all rat kidneys examined.

Statistical analysis

Data are presented as mean ± SEM. Differences between the groups were analyzed with the t test or the Mann–Whitney U test. The correlations between capillary density and glomerular maturity or tubulointerstitial injury were assessed using Spearman's correlation analysis. A p value < 0.05 was considered significant. Statistical analyses were performed with SPSS ver. 16.0 for Windows software (SPSS Inc., Chicago, IL).

Results

Renal histological alterations

Enalapril-treated rat kidneys showed widespread tubular, glomerular, and vascular abnormalities compared to those in control kidneys (Fig. 1). Hematoxylin and eosin kidney staining demonstrated a higher grade of tubular injury (Fig. 1a) and a lower grade of glomerular maturation that those in enalapril-treated rats (Fig. 1d) ($P < 0.05$ for both). The abnormal wall thickening of intrarenal vessels was also pronounced in the enalapril-treated kidneys compared to that in the control kidneys (Fig. 1g, h).

Changes in glomerular and peritubular capillary density

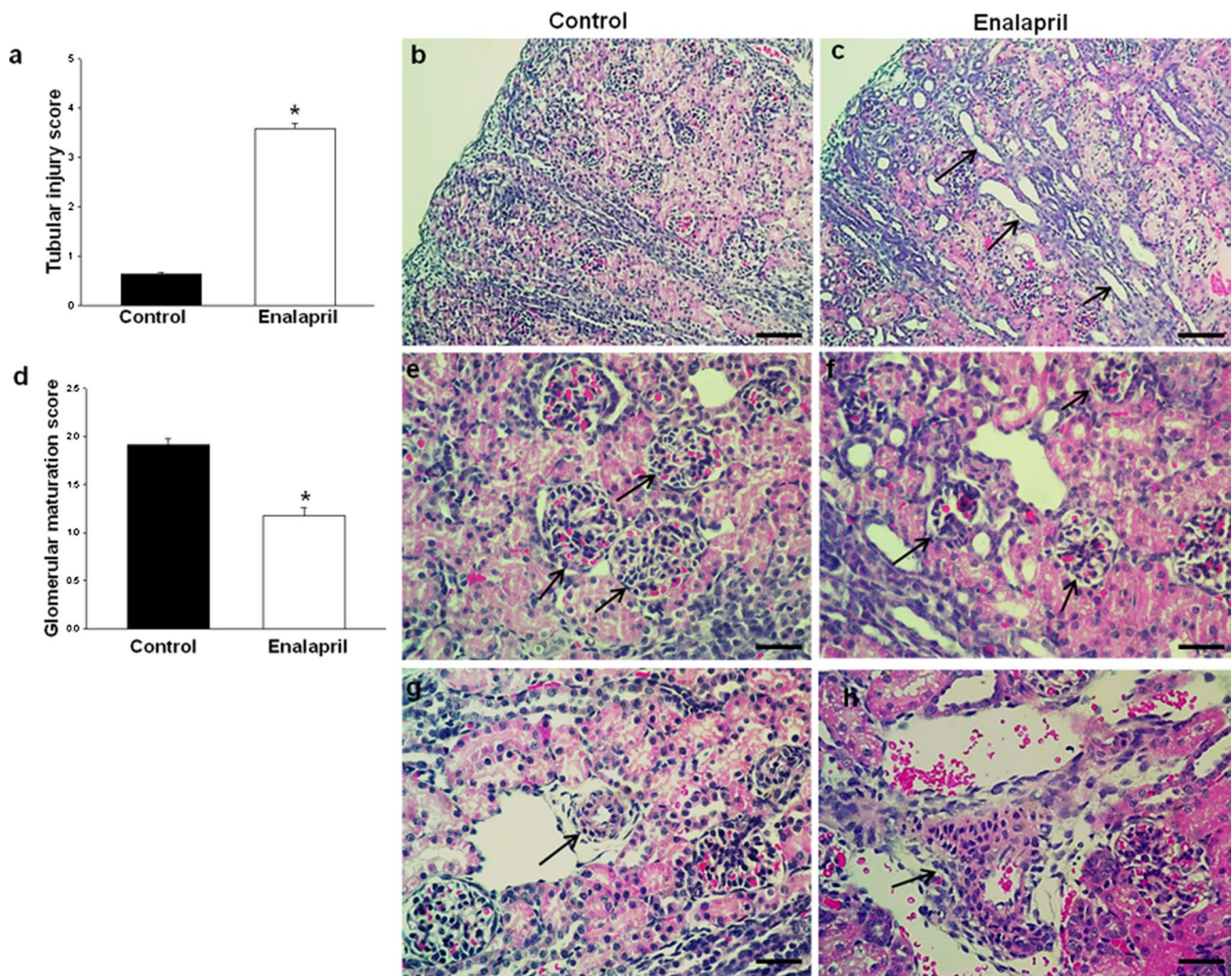The glomerular and peritubular capillaries detected by JG12 immunohistochemical staining were arranged

Fig. 1 Renal histological changes in 8-day-old rats. **a** Enalapril-treated rat kidneys shows a higher tubular injury score compared to that of the control rat kidney ($^{*}P < 0.05$ vs. control, t test). **b** Control rat kidney. **c** Striking tubular dilatation (arrows) was observed in the enalapril-treated group. **d** The enalapril-treated kidneys had less mature glomeruli than that in control rats ($^{*}P < 0.05$ vs. controls, t test). **e** Nearly mature glomeruli in the control rat kidney (arrows). **f** Glomeruli in the early stages of development in the enalapril-treated rat kidney (arrows). **g** Normal vessel in a control rat kidney (arrow) (**h**) Abnormal vascular wall thickening (arrow) in enalapril-treated rat kidney (Black bar, control rats; white bar, enalapril-treated rats) (n = 10 for each group) (Hematoxylin and eosin stain; **b, c** ×200, bar = 200 μm; **e–h** ×400, bar = 100 μm)

regularly, with a uniform size and shape in control rats (Fig. 2a, c). In contrast, JG12-positive capillaries decreased distinctly in enalapril-treated rat kidneys (Fig. 2b, d). A significant decrease in both glomerular and peritubular JG12 staining was detected in the enalapril-treated rats ($P < 0.05$) (Fig. 2e). In addition, the number of JG12-positive capillary endothelial cells was positively correlated with the glomerular maturation grade (r = 0.948, $P < 0.001$; Fig. 2f) and negatively correlated with the score of tubular injury (r = − 0.842, $P < 0.05$; Fig. 2g).

Ang-1, Ang-2, and Tie-2 expression

Ang-1 expression decreased significantly after enalapril treatment at postnatal day 8. Ang-1/β-actin protein expression decreased in the enalapril-treated group compared to that in the control ($P < 0.05$) (Fig. 3a). Ang-1 expression was high within the tubular epithelial cells and some glomeruli in the cortex of control kidneys (Fig. 3b) but was rarely detected in tubules and glomeruli in the enalapril-treated group (Fig. 3c). In contrast, Ang-2 and Tie-2
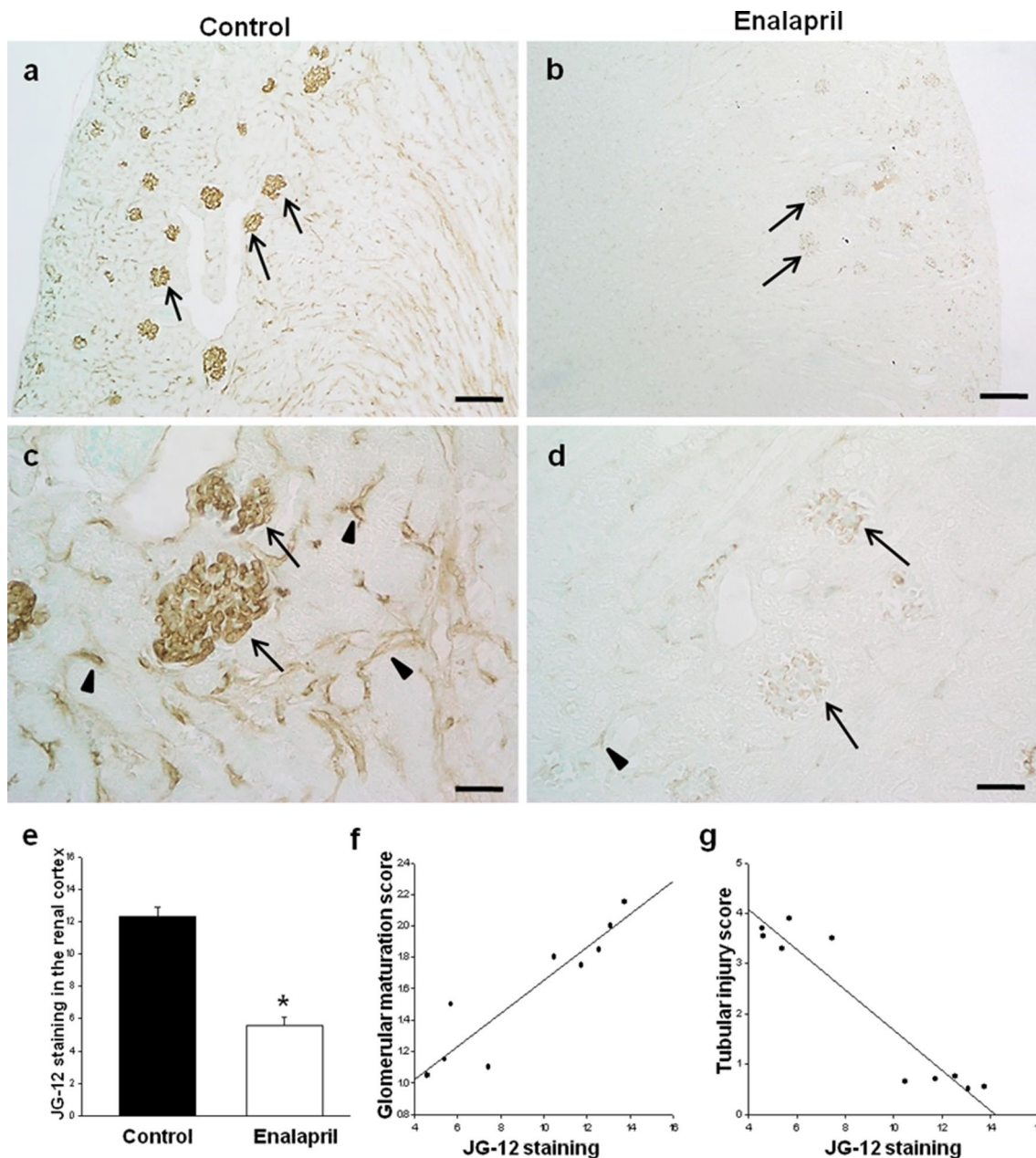
Fig. 2 JG12 expression in the capillary endothelium. **a, c** Glomerular (arrows) and peritubular capillaries (arrowheads) in the control rat kidney. **b, d** Glomerular (arrows) and peritubular capillaries (arrowhead) in the enalapril-treated rat kidney. **e** Capillary density decreased significantly in the enalapril-treated group (*$P < 0.05$ vs. controls, t test). The number of JG12-positive capillary endothelial cells was positively correlated with the stage of glomerular maturation (**f**) (r = 0.948, $P < 0.001$, Spearman's correlation test) and negatively correlated with the grade of tubular injury (**g**) (r = − 0.842, $P < 0.05$, Spearman's correlation test) (Black bar, control rats; white bar, enalapril-treated rats) (n = 10 for each group) (**a, b** ×100, bar = 400 μm; **c, d** ×400, bar = 100 μm)

expression increased significantly following the enalapril treatment. The immunoblots showed that the Ang-2/β-actin (Fig. 3d) and Tie-2/β-actin (Fig. 3g) protein expression increased significantly in the enalapril-treated group ($P < 0.05$). Ang-2 expression was strong in dilated tubular cells and some glomeruli of enalapril-treated rat kidneys

compared with that in control kidneys (Fig. 3e, f). Tie-2 expression was rarely detected in the cortex of control kidneys (Fig. 3h); however, it was more clearly detectable within glomeruli and peritubular capillaries of enalapril-treated rat kidneys (Fig. 3i).
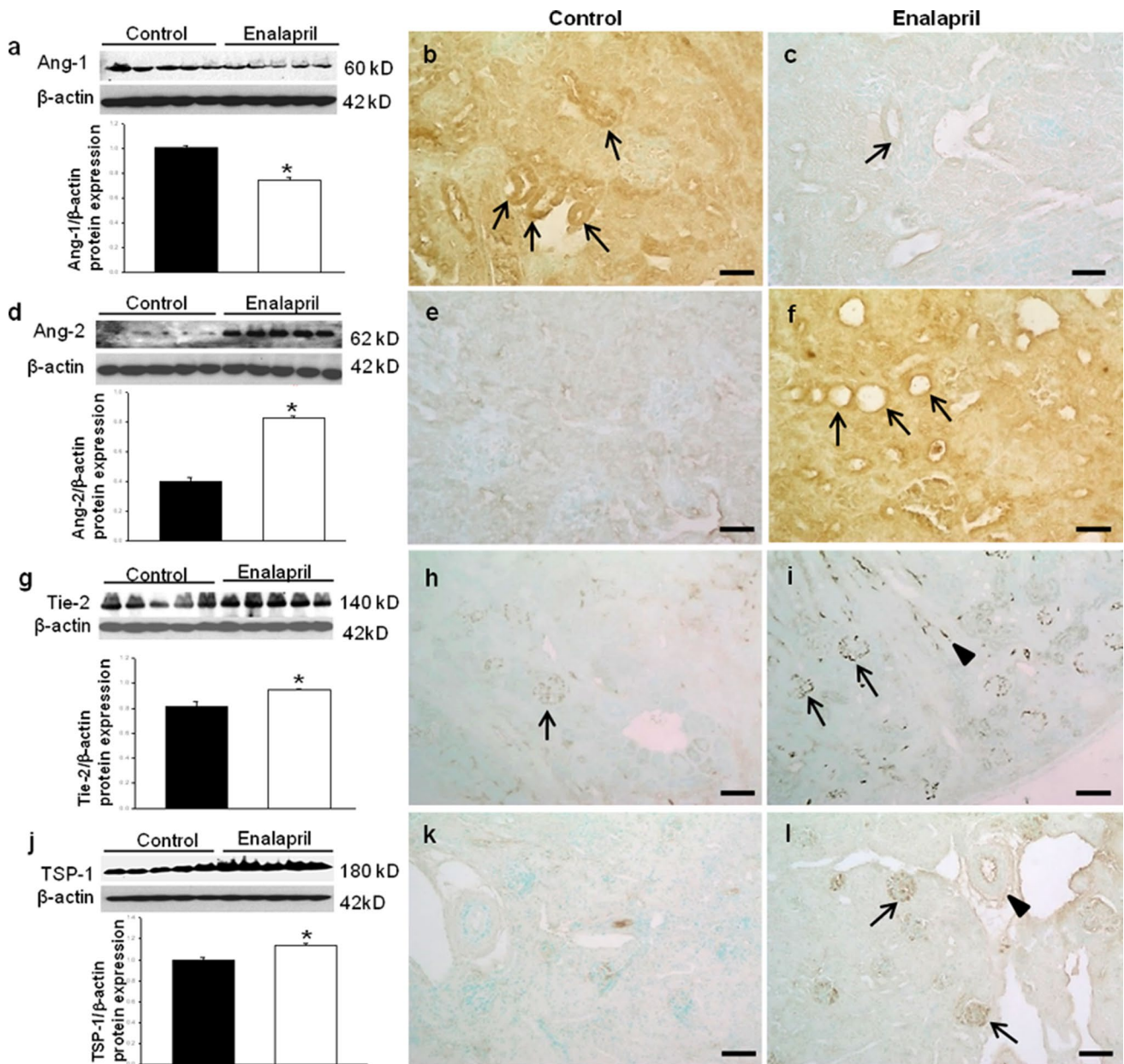
Fig. 3 Intrarenal expression of pro- and anti-angiogenic factors in 8-day-old rats. (**a–c**) Angiopoietin (Ang)-1. **d–f** Ang-2. **g–i** Tie-2. **j–l** Thrombospondin (TSP)-1 (**a, d, g,** and **j**, immunoblots; black bar, control rats; white bar, enalapril-treated rats; *P < 0.05 vs. controls; **a, d** t test; **g, j** Mann–Whitney U test) (**b, e, h, k**, control rat kidney; **c,** **f, i, l**, enalapril-treated rat kidney) (n = 10–12 for each group) (**b, c, f**; arrows, tubular expression; ×400, bar = 100 µm) (**h, i, l**; arrows, glomerular expression; arrowheads; peritubular capillary (**i**) or vascular (**l**) expression; ×200, bar = 200 µm)

TSP-1 expression

TSP-1/β-actin protein expression increased in the enalapril-treated group, compared to that in the control group (P < 0.05) (Fig. 3j). TSP-1 expression was scarcely observed in the cortex of the control kidneys (Fig. 3k), whereas it was clearly detected in glomeruli and some vascular walls in the enalapril-treated group (Fig. 3l).

Discussion

We demonstrated that treatment with enalapril for the first 7 days after birth reduced glomerular and peritubular capillary density in neonatal rat kidneys. This finding was associated with a decrease in glomerular maturity and an increase in tubular dilatation and atrophy. In addition, the expression of Ang-1 decreased, whereas the activities of Ang-2, Tie-2,

and TSP-1 increased after enalapril treatment in developing rat kidneys. Our findings suggest that an imbalance in the signaling interactions between pro- and anti-angiogenic factors have a specific role in the loss of capillaries in the developing rat kidney after inhibiting the ACE.

The RAS plays a significant role in renal vascularization and nephrogenesis (Sequeira-Lopez and Gomez 2011). Inhibiting the angiotensin II type 1 receptor in the developing rat arrests nephrovascular maturation and renal growth (Tufro-McReddie et al. 1995). Targeted inactivation of ACE gene induces remarkable renal defects in adult mice, consisting of reduced numbers of thickened, hypercellular renal arterioles, tubular dilatation, papillary atrophy, and interstitial fibrosis (Niimura et al. 1995). ACE is found in endothelial precursors invading the inferior cleft of the S-shaped body, glomerular capillaries, proximal tubules, peritubular capillaries, and corticomedullary arteries (Mizuiri and Ohashi 2015). Establishment of the S-shaped glomerulus appears to be crucial for glomerular vascularization, because endothelial cell precursors occupy the lower cleft and form the glomerular capillaries at this phase (Sequeira-Lopez and Gomez 2011). We postulated that inhibiting ACE may induce capillary loss as well as glomerular immaturity and tubular injury. Indeed, neonatal ACE inhibition in rats reduced capillary density and glomerular maturity and increased tubular dilatation and atrophy.

We detected JG12 to assess changes in capillary density and renal angiogenesis. JG12 is a specific marker for the blood vessel endothelium and distinctively expressed by the endothelial cells of glomeruli and tubulointerstitial vessels in the kidney (Matsui et al. 2003; Sun et al. 2012). JG12 is more discriminatory than other endothelial markers, such as CD34, CD31, which are expressed in both lymphatic and blood vessels (Matsui et al. 2003). Sun et al. (2012) showed a substantial reduction in peritubular capillary density detected by JG12 immunostaining after unilateral ureteral obstruction surgery. JG12-positive peritubular capillaries were markedly diminished from some regions with significant interstitial expansion and tubular atrophy, as they were changed by fibrotic elements. In the present study, a significant decrease in both glomerular and peritubular JG12 expression was detected after enalapril treatment for up to 1 week after birth. It is likely that the renal capillary loss in the enalapril-treated rats resulted in poor oxygen supply and nutrients to the glomerular and tubular cells, which may have impaired postnatal renal maturation. Supporting this finding, capillary density was negatively correlated with glomerular immaturity and tubulointerstitial injury. Given that kidney vascularization and nephrogenesis are matched synchronized processes (Sequeira-Lopez and Gomez 2011), the disturbance in maintaining glomerular and peritubular capillaries may partly explain the renal structural abnormalities caused by inhibiting ACE in the developing rat kidney.

Madsen et al. (2010) observed significant deterioration in renal microvasculature and vasa recta bundles in candesartan (angiotensin II type 1 receptor blocker)-treated animals for up to 2 weeks after birth, accompanied by inhibited VEGF, Ang-1, Ang-2, and Tie-2. Medullary abnormalities, including hypoplasia of the papilla and thickening of vessels, and a marked reduction in renal blood flow also occurred. Those authors suggested that angiotensin II increases postnatal growth of postglomerular capillaries and organization of vasa recta bundles and that postnatal renal development is contingent on proper regulation of angiogenesis. In the present study, treatment with ACE inhibitor for 1 week after birth led to a decrease in Ang-1 expression and enhanced levels of Ang-2, Tie-2, and TSP-1. The reasons for the dissimilar results may be attributable to differences in time points and the level of blockade of angiotensin II action. ACE inhibition prevents the degradation of bradykinin as well as the formation of angiotensin II. Blockade of ACE activity also augments circulating levels of the bioactive peptide angiotensin-(1–7) (Gallagher et al. 2011). Several in vitro and in vivo models of angiogenesis have been shown to the anti-angiogenic properties of angiotensin-(1–7) (Gallagher et al. 2011; Pei et al. 2016). Therefore, elevated bradykinin level and an altered balance between angiotensin II and angiotensin-(1–7) might be associated with the mechanism of action of ACE inhibition (Simões et al. 2016).

Angs are a family of growth factors that play a vital role in vascular development (Parikh 2017). Ang-1 binds to and tyrosine-phosphorylates endothelial Tie-2, producing increased survival and cell–cell stabilization (Woolf et al. 2009). Ang-1 null embryos initiate blood vessel formation, but the vascular endothelium fails to remodel (Suri et al. 1996). Ang-1 therapies reduce peritubular capillary loss in adult models of tubulointerstitial disease (Long et al. 2008). In contrast, podocyte-specific overexpression of Ang-2 induces glomerular endothelial apoptosis, decreases nephrin expression, and augments albuminuria (Davis et al. 2007). Contrary to Ang-1/Tie-2 signaling, activation of the Ang-2/Tie-2 signaling leads to loss of cell–cell contacts and vascular destabilization (Biel and Siemann 2016). Blockade of the Ang-2/Tie-2 signaling by using a specific inhibitor of Ang-2 obviously promoted angiogenesis (Yan et al. 2017). In this study, enalapril treatment for the first 7 days after birth reduced Ang-1 expression but increased Ang-2 and Tie-2 activities in developing rat kidneys. These findings agree with the regression of glomerular and peritubular capillaries and may have contributed to impaired vascular expansion at a later kidney developmental stage. The Ang system may play an important role in renovascular maturation and microvascular injury after inhibiting ACE during postnatal kidney development.

In parallel, TSP-1 expression was upregulated in the enalapril-treated rat kidneys compared to that in the

controls. TSP-1 directly initiates endothelial cell apoptosis and plays a key role as a natural inhibitor of angiogenesis (Zhang and Lawler 2007). As an endogenous activator of TGF-β, it also prevents kidney repair (Isenberg et al. 2009). Using a ligature relief model after unilateral ureteral obstruction, Bige et al. (2012) showed that kidney repair involves early downregulation of TSP-1 and TGF-β1 expression followed by regeneration of peritubular capillaries and proximal tubules. The proapoptotic action of TSP-1 during ischemia/reperfusion injury was also detected in TSP-1 null mice (Thakar et al. 2005). The present data showed that enalapril treatment during the first 7 postnatal days increased TSP-1 expression in neonatal rat kidneys. Overexpression of TSP-1 suggests a deleterious effect of TSP-1 on preservation of glomerular and tubular structures as well as the capillary network. TSP-1 may favor cell apoptosis and anti-angiogenic environment in enalapril-treated rat kidneys, leading to loss of kidney microvasculature.

We have previously shown improper VEGF-A/VEGF receptor signaling during neonatal inhibition of ACE (Yim et al. 2016). VEGF-A protein expression increased in enalapril-treated rat kidneys. However, the major angiogenic VEGF receptor-2 activity remained unchanged, although the anti-angiogenic VEGF receptor-1 expression decreased. Here we further investigated the role of the Ang system and TSP-1 in renovascular injury in the same model. We found that these molecules contribute to capillary loss in enalapril-treated rats through reduced Ang-1 expression and enhanced activation of Ang-2, Tie-2, and TSP-1. Notably, the biologic effects of the Angs rely on ambient levels of VEGF-A. Sprouting angiogenesis follows destabilization of blood vessels by Ang-2 in a VEGF-rich state, whereas Ang-2 induces endothelial cell death and vessel regression in a VEGF-depleted environment (Augustin et al. 2009; Jin and Patterson 2009). The enhanced TSP-1 expression hampers VEGF-mediated endothelial cell proliferation and independently provokes endothelial cell apoptosis in renal fibrosis models (Hugo et al. 2002). In our experimental model, the angiogenic response could initially occur due to increased survival from the VEGF-A/VEGF receptor-1 signaling in response to kidney injury by enalapril. The simultaneous downregulation of Ang-1 and upregulation of Ang-2, Tie-2, and TSP-1 may have destabilized kidney microvessels and suppressed VEGF-A-mediated endothelial cell proliferation. This imbalanced environment between pro- and anti-angiogenic factors may have led to an antiangiogenic condition that favored loss of glomerular and peritubular capillaries. Collectively, a cooperated interplay of several key players, such as Ang-1, Ang-2, Tie-2, TSP-1, and VEGF-A/VEGF receptor signaling, can participate in microvascular injury associated with tubular and glomerular changes in developing rat kidney after inhibiting ACE.

Some limitations of our study should be discussed. A stereological quantification of total capillary length or volume and glomerular number or size in kidney tissue may have given greater power to this study. The direct effects of candidate molecules in angiotensin II-mediated postnatal angiogenesis further support a primary role for these molecular mechanisms during the normal renovascular growth response to angiotensin II. The potential involvement of angiogenesis-regulating factors in this model needs to be further validated by strategies such as specific overexpression or deletion of the appropriate genes.

In conclusion, our data provide new clues to understand the mechanisms of impaired vascular development after inhibiting ACE in neonatal rat kidney. Renal structural deterioration can be associated with glomerular and peritubular capillary rarefaction. The Ang signaling system and TSP-1 appear to be important mediators and may be relevant in the pathogenesis of microvascular injury after RAS blockade in the developing kidney.

Author Contributions KH Yoo and HE Yim contributed to study design, data analysis, data interpretation, and manuscript writing. ES Bae contributed to data acquisition and data analysis. YS Hong contributed to data interpretation and manuscript writing. All authors approved the final version of the manuscript.

Compliance with ethical standards

Conflict of interest The authors have declared no conflicts of interest.

Ethical approval All applicable international, national, and/or institutional guidelines for the care and use of animals were followed. This article does not contain any studies with human participants performed by any of the authors.

References

Abrahamson DR (2009) Development of kidney glomerular endothelial cells and their role in basement membrane assembly. Organogenesis 5:275–287

Augustin HG, Koh GY, Thurston G, Alitalo K (2009) Control of vascular morphogenesis and homeostasis through the angiopoietin-Tie system. Nat Rev Mol Cell Biol 10:165–177

Biel NM, Siemann DW (2016) Targeting the Angiopoietin-2/Tie-2 axis in conjunction with VEGF signal interference. Cancer Lett 380:525–533

Bige N, Shweke N, Benhassine S, Jouanneau C, Vandermeersch S, Dussaule JC, Chatziantoniou C, Ronco P, Boffa JJ (2012) Thrombospondin-1 plays a profibrotic and pro-inflammatory role during ureteric obstruction. Kidney Int 81:1226–1238

Chen J, Chen JK, Conway EM, Harris RC (2013) Survivin mediates renal proximal tubule recovery from AKI. J Am Soc Nephrol 24:2023–2033

Davis B, Dei Cas A, Long DA, White KE, Hayward A, Ku CH, Woolf AS, Bilous R, Viberti G, Gnudi L (2007) Podocyte-specific expression of angiopoietin-2 causes proteinuria and apoptosis of glomerular endothelia. J Am Soc Nephrol 18:2320–2329

de Almeida LF, Francescato HDC, da Silva CGA, Costa RS, Coimbra TM (2017) Calcitriol reduces kidney development disorders in rats provoked by losartan administration during lactation. Sci Rep 7:11472

Eremina V, Sood M, Haigh J, Nagy A, Lajoie G, Ferrara N, Gerber HP, Kikkawa Y, Miner JH, Quaggin SE (2003) Glomerular-specific alterations of VEGF-A expression lead to distinct congenital and acquired renal diseases. J Clin Invest 111:707–716

Friberg P, Sundelin B, Bohman SO, Bobik A, Nilsson H, Wickman A, Gustafsson H, Petersen J, Adams MA (1994) Renin-angiotensin system in neonatal rats: induction of a renal abnormality in response to ACE inhibition or angiotensin II antagonism. Kidney Int 45:485–492

Gallagher PE, Cook K, Soto-Pantoja D, Menon J, Tallant EA (2011) Angiotensin peptides and lung cancer. Curr Cancer Drug Targets 11:394–404

Gnudi L, Benedetti S, Woolf AS, Long DA (2015) Vascular growth factors play critical roles in kidney glomeruli. Clin Sci 129:1225–1236

Gomez RA, Lynch KR, Chevalier RL, Everett AD, Johns DW, Wilfong N, Peach MJ, Carey RM (1988) Renin and angiotensinogen gene expression and intrarenal renin distribution during ACE inhibition. Am J Physiol 254:F900-906

Gomez RA, Norwood VF, Tufro-McReddie A (1997) Development of the kidney vasculature. Microsc Res Tech 39:254–260

Guo N, Krutzsch HC, Inman JK, Roberts DD (1997) Thrombospondin 1 and type I repeat peptides of thrombospondin 1 specifically induce apoptosis of endothelial cells. Cancer Res 57:1735–1742

Hartman HA, Lai HL, Patterson LT (2007) Cessation of renal morphogenesis in mice. Dev Biol 310:379–387

Herzlinger D, Hurtado R (2014) Patterning the renal vascular bed. Semin Cell Dev Biol 36:50–56

Hugo C, Kang DH, Johnson RJ (2002) Sustained expression of thrombospondin-1 is associated with the development of glomerular and tubulointerstitial fibrosis in the remnant kidney model. Nephron 90:460–470

Isenberg JS, Martin-Manso G, Maxhimer JB, Roberts DD (2009) Regulation of nitric oxide signaling by thrombospondin 1: implications for anti-angiogenic therapies. Nat Rev Cancer 9:182–194

Jin SW, Patterson C (2009) The opening act: vasculogenesis and the origins of circulation. Arterioscler Thromb Vasc Biol 29:623–629

Kida Y, Tchao BN, Yamaguchi I (2014) Peritubular capillary rarefaction: a new therapeutic target in chronic kidney disease. Pediatr Nephrol 29:333–342

Kolatsi-Joannou M, Li XZ, Suda T, Yuan HT, Woolf AS (2001) Expression and potential role of angiopoietins and Tie-2 in early development of the mouse metanephros. Dev Dyn 222:120–126

Long DA, Price KL, Ioffe E, Gannon CM, Gnudi L, White KE, Yancopoulos GD, Rudge JS, Woolf AS (2008) Angiopoietin-1 therapy maintains kidney peritubular capillaries but enhances fibrosis and inflammation after folic acid-induced acute renal failure. Kidney Int 74:300–309

Madsen K, Marcussen N, Pedersen M, Kjaersgaard G, Facemire C, Coffman TM, Jensen BL (2010) Angiotensin II promotes development of the renal microcirculation through AT1 receptors. J Am Soc Nephrol 21:448–459

Maisonpierre PC, Suri C, Jones PF, Bartunkova S, Wiegand SJ, Radziejewski C, Compton D, McClain J, Aldrich TH, Papadopoulos N, Daly TJ, Davis S, Sato TN, Yancopoulos GD (1997) Angiopoietin-2, a natural antagonist for Tie2 that disrupts in vivo angiogenesis. Science 277:55–60

Matsui K, Nagy-Bojarsky K, Laakkonen P, Krieger S, Mechtler K, Uchida S, Geleff S, Kang DH, Johnson RJ, Kerjaschki D (2003) Lymphatic microvessels in the rat remnant kidney model of renal fibrosis: aminopeptidase p and podoplanin are discriminatory markers for endothelial cells of blood and lymphatic vessels. J Am Soc Nephrol 14:1981–1989

Mizuiri S, Ohashi Y (2015) ACE and ACE2 in kidney disease. World J Nephrol 4:74–82

Niimura F, Labosky PA, Kakuchi J, Okubo S, Yoshida H, Oikawa T, Ichiki T, Naftilan AJ, Fogo A, Inagami T et al (1995) Gene targeting in mice reveals a requirement for angiotensin in the development and maintenance of kidney morphology and growth factor regulation. J Clin Invest 96:2947–2954

Parikh SM (2017) The angiopoietin-Tie2 signaling axis in systemic inflammation. J Am Soc Nephrol 28:1973–1982

Pei N, Wan R, Chen X, Li A, Zhang Y, Li J, Du H, Chen B, Wei W, Qi Y, Zhang Y, Katovich MJ, Sumners C, Zheng H, Li H (2016) Angiotensin-(1–7) decreases cell growth and angiogenesis of human nasopharyngeal carcinoma xenografts. Mol Cancer Ther 15:37–47

Sequeira-Lopez ML, Gomez RA (2011) Development of the renal arterioles. J Am Soc Nephrol 22:2156–2165

Sequeira-Lopez ML, Nagalakshmi VK, Li M, Sigmund CD, Gomez RA (2015) Vascular versus tubular renin: role in kidney development. Am J Physiol Regul Integr Comp Physiol 309:R650-657

Simões E, Silva AC, Teixeira MM (2016) ACE inhibition, ACE2 and angiotensin-(1–7) axis in kidney and cardiac inflammation and fibrosis. Pharmacol Res 107:154–162

Sun D, Wang Y, Liu C, Zhou X, Li X, Xiao A (2012) Effects of nitric oxide on renal interstitial fibrosis in rats with unilateral ureteral obstruction. Life Sci 90:900–909

Suri C, Jones PF, Patan S, Bartunkova S, Maisonpierre PC, Davis S, Sato TN, Yancopoulos GD (1996) Requisite role of angiopoietin-1, a ligand for the TIE2 receptor, during embryonic angiogenesis. Cell 87:1171–1180

Sutherland MR, Gubhaju L, Moore L, Kent AL, Dahlstrom JE, Horne RS, Hoy WE, Bertram JF, Black MJ (2011) Accelerated maturation and abnormal morphology in the preterm neonatal kidney. J Am Soc Nephrol 22:1365–1374

Takaori K, Nakamura J, Yamamoto S, Nakata H, Sato Y, Takase M, Nameta M, Yamamoto T, Economides AN, Kohno K, Haga H, Sharma K, Yanagita M (2016) Severity and frequency of proximal tubule injury determines renal prognosis. J Am Soc Nephrol 27:2393–2406

Thakar CV, Zahedi K, Revelo MP, Wang Z, Burnham CE, Barone S, Bevans S, Lentsch AB, Rabb H, Soleimani M (2005) Identification of thrombospondin 1 (TSP-1) as a novel mediator of cell injury in kidney ischemia. J Clin Invest 115:3451–3459

Tufro-McReddie A, Romano LM, Harris JM, Ferder L, Gomez RA (1995) Angiotensin II regulates nephrogenesis and renal vascular development. Am J Physiol 269:F110-115

Tufro-McReddie A, Norwood VF, Aylor KW, Botkin SJ, Carey RM, Gomez RA (1997) Oxygen regulates vascular endothelial growth factor-mediated vasculogenesis and tubulogenesis. Dev Biol 183:139–149

Woolf AS, Gnudi L, Long DA (2009) Roles of angiopoietins in kidney development and disease. J Am Soc Nephrol 20:239–244

Yan ZX, Luo Y, Liu NF (2017) Blockade of angiopoietin-2/Tie2 signaling pathway specifically promotes inflammation-induced angiogenesis in mouse cornea. Int J Ophthalmol 10:1187–1194

Yim HE, Yoo KH, Bae IS, Jang GY, Hong YS, Lee JW (2009) Aldosterone regulates cellular turnover and mitogen-activated protein kinase family expression in the neonatal rat kidney. J Cell Physiol 219:724–733

Yim HE, Yoo KH, Bae ES, Hong YS, Lee JW (2016) Impaired angiogenesis in the enalapril-treated neonatal rat kidney. Korean J Pediatr 59:8–15

Zhang X, Lawler J (2007) Thrombospondin-based antiangiogenic therapy. Microvasc Res 74:90–99