Roles of Angiopoietins in Kidney Development and Disease

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ABSTRACT

Angiopoietins are a family of growth factors, the best studied being angiopoietin 1 (Ang-1), which binds to and tyrosine-phosphorylates endothelial Tie-2, causing enhanced survival and cell–cell stabilization. Ang-2 and Tie-1 downregulate Ang-1–induced Tie-2 signaling, and angiopoietin actions are further modified by vascular endothelial growth factor A and integrins. Metanephric capillaries express Tie genes, whereas metanephric mesenchyme, maturing tubules, and mature podocytes express Ang-1. Ang-1 null embryos begin to form blood vessels, but subsequent vascular remodeling fails, and analyses of chimeric wild-type/Tie null mutant embryos show that Tie genes are needed for renal endothelial survival. Ang-2 is transiently expressed in renal arterial smooth muscle and mesangial cells, and tubules around adult vasa rectae express Ang-2. Ang-2 null mice have increased pericytes around kidney cortical peritubular capillaries, perhaps an indirect consequence of upregulated Tie-2 signaling. Ang-1 therapies attenuate peritubular capillary loss in adult models of tubulointerstitial disease, although, in one study, this was accompanied by enhanced inflammation and fibrosis. Podocyte-directed Ang-2 transgenic overexpression causes glomerular endothelial apoptosis, downregulated nephrin expression, and increased albuminuria, and glomerular Ang-2 is upregulated in hyperglycemic and immune-mediated glomerulopathies. Thus, angiopoietins affect podocyte as well as glomerular endothelial biology, and imbalanced angiopoietin signaling contributes to glomerular pathobiology.


Angiopoietins are a family of vascular growth factors, the best-studied being angiopoietin 1 (Ang-1) and Ang-2.1–4 During normal development, they are considered critical for vascular differentiation through angiogenesis, the process of growth and remodeling of existing vessels; they are also involved in the maintenance and turnover of blood vessels in late gestation and in mature animals.1–3,5 Ang-1 and Ang-2 are ligands for the Tie-2 (tyrosine kinase with Ig and EGF homology domain 2) receptor tyrosine kinase, which is characteristically expressed by blood endothelial cells. Ang-1 oligomers and multimers bind to and tyrosine-phosphorylate Tie-2 through their COOH-terminal fibrinogen-like domains, causing enhanced endothelial survival and endothelial cell–cell stabilization.6 Furthermore, Tie-2 activation indirectly recruits supporting perivascular cells (pericytes and smooth muscle cells), likely through the action of paracrine factors released by endothelia themselves; this is required for stabilization of newly formed vessels. Ang-2 is a natural antagonist of Ang-1, an effect mediated by the Ang-2 competitively inhibiting binding of Ang-1 to Tie-2; however, other data suggest that Ang-2 may, in certain situations, also activate Tie-2.7,8 Less is known about the homologous receptor Tie-1, although it is widely expressed by developing endothelia and it downregulates intracellular signaling triggered by angiopoietin-induced Tie-2 phosphorylation,9 thereby providing a fine-tuning mechanism for signaling through this receptor.

Importantly, the in vivo biologic effects of the angiopoietins also depend on ambient levels of vascular endothelial growth factor A (VEGF-A); for example, with respect to the actions of Ang-2, vessel regression occurs when VEGF-A is lacking, whereas vessel destabilization is followed by angiogenesis when the local milieu is rich in VEGF-A.10 Integrins such as α5β1 can upregulate Ang-1/Tie-2 signaling and may even facilitate angiopoietin bioactivity in the absence of Tie-2 through outside-in signaling after Ang-1 binds to and activates integrins.11 Tie-2 is not exclusively expressed by blood endothelia, suggesting that other cell types may be direct targets for the angiopoietins. These include normal epithelia,12 with Tie immunolocalizing in female reproductive tract cilia; differentiating lymphatic endothelial cells13; and...
certain (nonendothelial) tumor-associated cells.

Angiopoietins also have potentially complex direct and indirect effects on inflammatory responses. For example, Ang-1 inhibits TNF-α–induced leukocyte capillary transmigration, whereas Ang-2 destabilizes endothelial cell–cell junctions and enhances leakage of inflammatory cells and also sensitizes endothelia to TNF-α. Within endothelia, Ang-2 itself is stored in and can be rapidly released from Weibel-Palade bodies, this source of the factor may therefore be implicated in vascular inflammatory responses. Monocytes/macrophages and neutrophils can express Tie-2 and be recruited by the angiopoietins.

Hypoxia, adrenocorticotropin, glucose, and TNF-α upregulate Ang-2 expression, and sonic hedgehog, a secreted growth factor, upregulates expression of both Ang-1 and Ang-2. Tie-2 gene expression is upregulated in low oxygen tensions through hypoxia-inducible factor–induced transcriptional activation.

ANGIOPOIETINS IN KIDNEY DEVELOPMENT

The metanephros is the mammalian precursor of the mature kidney. Ang-1, Ang-2, Tie-1, and Tie-2 all are expressed from the inception of the mouse metanephros, when the organ simply comprises ureretic bud epithelia and metanephric mesenchyme. In mice, levels of Ang-1, Ang-2, Tie-1, and Tie-2 transcripts peak soon after birth, and all four genes remain expressed in the adult kidney. Phosphorylated Tie-2 can be detected in the late-gestation mouse kidney and is also detected postnatally, through to adulthood. Mouse metanephric interstitial and glomerular capillaries express Tie genes, whereas Ang-1 is expressed by nephrogenic mesenchyme, as well as by differentiating tubule epithelium and by differentiating and mature podocytes, and human renal mesenchyme also expresses Ang-1. Ang-2 is transiently expressed in renal arterial smooth muscle and mesangial cells, but, in adulthood, Ang-2 is expressed in tubules near vasa rectae. All of these observations are consistent with hypotheses that angiopoietins play multiple roles in renovascular maturation, perhaps in parallel with VEGF-A, which has established effects on metanephric endothelial differentiation and survival.

Ang-1 null embryos begin to form blood vessels, but normal vascular remodeling fails to occur; however, they die before the metanephros differentiates so are uninformative for studying kidney development. Exogenous recombinant Ang-1 does, however, enhance the growth of interstitial capillaries in mouse metanephric organ culture, and the factor enhances transendothelial electrical resistance in monolayer cultures of glomerular endothelia. These effects are consistent with reports that Ang-1 enhances survival of nonrenal endothelia and also that the factor stabilizes endothelial cell–cell interactions.

Within the metanephric mesenchyme are found clusters of Tie-1–expressing cells. Whether they are angioblasts that have migrated into the initiating kidney alongside the incoming ureteric bud or endothelial precursors that have differentiated de novo within the mesenchyme by vasculogenesis is unknown. Using transplantation experiments with the just-formed metanephros, both glomerular and interstitial capillaries arise from transplanted material, suggesting that Tie-expressing cells are renal capillary precursors. Other studies showed that there are subsets of VEGF-A receptor–expressing cells within metanephric mesenchyme, and these may be the same cells that express Tie-1. In chimeric mice, composed of wild-type cells and those that lack Tie-1 and Tie-2, mutant cells are present in embryonic (nonrenal) vessels when they initiate, but mutant cells cannot be detected in renal blood vessels in late gestation, suggesting that Ang/Tie signaling is required for survival of metanephric endothelia. Genetic deficiency of the Wnt-4 growth factor causes a reduction of metanephric Tie-1–expressing interstitial capillaries, whereas hypoxic culture of whole metanephroi upregulates Tie-1 expression in peritubular cells; whether these effects are mediated by direct effects on endothelial precursors or by indirect actions (tubular-endothelial cross-talk) is not known. It is of interest to note, however, that normal embryonic kidneys are most likely hypoxic in vivo, as assessed by immunodetection of hypoxia-inducible factor 1α and 2α proteins.

Ang-2 null mutants die soon after birth with chylosus ascites, and neonates display dysmorphism of cortical peritubular capillaries. This observation is consistent with the idea that Ang-2 downregulates Ang-1 signaling in blood endothelia, which themselves then stabilize surrounding smooth muscle cells/pericytes by releasing trophic factors. Indeed, separation of endothelium and supporting cells is a feature of embryos in which either Ang-1 has been deleted or Ang-2 has been overexpressed.

TRANSGENIC MANIPULATION OF ANG-2 EXPRESSION IN THE KIDNEY

We have begun to explore the in vivo actions of the angiopoietins in glomerular biology by generating transgenic mice with inducible overexpression of Ang-2. We used a reverse tetracycline-controlled transcriptional activator (rtTA) activated by doxycycline and elected to drive expression of rtTA using the Podocin promoter, resulting in podocyte-specific activation. We considered that the adult podocyte was an appropriate glomerular cell type to use as a source for the angiopoietins because they normally express Ang-1 and Ang-2. Podocin-rtTA mice were bred with mice containing a response element driving the Ang-2 gene, resulting in tightly controlled overexpression of this factor. From 5 wk after Ang-2 transgene expression was induced in adults, there were significant increases in albuminuria and glomerular endothelial apoptosis, with significant decreases of both VEGF-A and nephrin proteins, respectively, critical for maintenance of glomerular endothelia and glomerular filtration barrier integrity.
were no changes of systemic BP or creatinine clearance, and podocytes were ultrastructurally intact as assessed by electron microscopy. In vitro, short-term exposure of isolated wild-type murine glomeruli to exogenous Ang-2 also led to decreased protein levels of VEGF-A.43

The increased in vivo apoptosis of glomerular endothelia was not an unexpected effect, consistent with the overexpressed Ang-2 accessing and directly altering the biology of these cells, antagonizing signaling of endogenous Ang-1; in fact, endothelial death may have been enhanced by VEGF-A downregulation in transgenic Ang-2–overexpressing glomeruli.43 The changes in podocyte gene expression might have resulted from altered signaling between endothelia and podocytes. Alternatively, Ang-2 might have direct effects on podocytes, perhaps by Tie-mediated or independent mechanisms (Figure 1). In this regard, one study immunolocalized Tie-2 on rat podocytes in vivo, as well as on glomerular endothelia.43 In addition, one can postulate direct effects of angiopoietins on podocytes by non–Tie-mediated pathways, for example mediated by integrins.11

ANGIOPOIETIN EXPRESSION IN ACQUIRED GLOMERULAR DISEASE

Diabetic nephropathy is the leading cause of end-stage renal failure in the Western world. An early sign is a small increase in the quantity of urinary protein, manifested by microalbuminuria, which correlates with and can predict the progression of renal damage and cardiovascular morbidity. Microalbuminuria in individuals with diabetic nephropathy is considered to arise from increased protein losses in the glomerular filtrate caused by defects in the filtration barrier, which separates the blood circulation from the urinary space. In human and animal diabetic nephropathy, glomerular expression of nephrin is downregulated, and a similar change occurred when Ang-2 overexpression was induced in transgenic (nondiabetic) mice. Experimental models of type 1 diabetes are associated with altered renal expression of angiopoietins. Rizkalla et al. reported that administration of streptozotocin, an islet toxin, to adult rats led to hyperglycemia within 1 wk, with increased albumin excretion, systemic hypertension, and nephromegaly at 4 and 8 wk. Whole-kidney Ang-1 and Ang-2 mRNA and protein levels rose at 4 wk, but at 8 wk, Ang-1 levels were lower than those in nondiabetic controls, whereas Ang-2 remained elevated. Ang-1 was immunolocalized in diabetic kidney tubules, whereas Ang-2 was prominent in glomerular endothelia and podocytes. Yamamoto et al. also reported upregulated Ang-2 in a model of streptozotocin-induced diabetic nephropathy in mice, and individuals with type 2 diabetes have elevated circulating Ang-2 levels. Glucose stimulates Ang-2 expression, providing one explanation for Ang-2 upregulation in diabetic nephropathy. Collectively, these observations are consistent with the contention that a decreased ratio of Ang-1/Ang-2 might play a role in the pathobiology of glomerular disease in diabetic nephropathy.

Angiopoietin expression has been investigated in other glomerular disease models. Yuan et al. found that, in a mouse model of anti–glomerular basement membrane glomerulonephritis, glomerular expression of Ang-1 was reduced and Ang-2 was increased, correlating with glomerular endothelial apoptosis and VEGF-A downregulation. In daunorubicin-induced glomerular disease, the appearance of glomeruloclesis correlated with a decreasing Ang-1/Ang-2 expression ratio, and Liang et al. reported that exposure of isolated podocytes to puromycin aminonucleoside led to decreased angiogenic activity of supernatants, along with decreased levels of Ang-1 and VEGF-A. Collectively, these observations are consistent with the contention that altered expression of angiopoietins might play roles in the pathobiology of glomerular disease associated with attrition of capillaries and proteinuria. In addition, the modulating effects of angiopoietins on inflammation, allowed to previously, may also be relevant to glomerulonephritides; however, to explore further the possible roles of angiopoietins in glomerular disease, one will

Figure 1. Putative actions of the angiopoietins within glomeruli. Angiopoietin growth factors within glomeruli may directly access endothelial cells and modify Tie-2 phosphorylation. As examples, Ang-1, normally expressed by podocytes, would may regulate Tie-2 signaling, causing enhanced endothelial survival and endothelial cell–cell stabilization. In glomerular disease, upregulated Ang-2 would antagonize these effects. At the same time, unknown secondary signals from endothelia stimulated by angiopoietins may alter podocyte gene expression and ultrastructure. Alternatively, one can hypothesize that angiopoietins have direct effects on podocytes, perhaps through binding to Tie-2 and/or integrins on podocytes themselves. Dashed lines represent yet-to-be proven pathways.
have to manipulate their levels in these same models. In this regard, some promising data were reported by Lee et al., who demonstrated that systemic delivery of cartilage oligomeric matrix protein–Ang-1 (COMP-Ang-1; a modified form of Ang-1) by adenoviral transduction of hepatocytes reduced renal fibrosis in db/db mice, a model of type 2 diabetes; however, this strategy also caused significant improvement in hyperglycemia, which could itself, at least partly, account for the amelioration of diabetes.

NEPHROPATHIES ASSOCIATED WITH TUBULOINTERSTITIAL LESIONS

In humans who have chronic kidney disease with moderately to severely reduced GFR, Futrakul et al. reported that circulating levels of VEGF-A and Ang-1 were decreased, whereas those of Ang-2 were elevated. This suggests that an “anti-angiogenic environment” exists in long-standing nephropathies. Indeed, a chronic loss of renal interstitial capillaries occurs in human nephropathies. In animal models, the situation seems more complex; for example, some mouse strains have a prolonged angiogenic response after subtotal nephrectomy, whereas rats seem more prone to lose interstitial capillaries after the same maneuver. Whereas angiopoietin expression has yet to be reported in these particular models, it has been studied in other kidney diseases that feature prominent tubulointerstitial lesions. Kim et al. performed unilateral ureteric obstruction in adult mice and noted that kidney Ang-1 levels fell. After a chimeric form of Ang-1, COMP-Ang-1, was transduced into the liver by an adenoviral vector, the result was increased kidney Tie-2 phosphorylation and preservation of peritubular capillaries in association with decreased macrophage numbers and decreased TGF-β expression. Long et al. used adenoviral delivery of Ang-1*, a more soluble form of Ang-1 with an NH2-terminal more closely resembling Ang-2, in a mouse model of folic acid–induced nephropathy. They found that when Ang-1* was administered a few days before the nephrotoxin, although the severity of acute renal failure was not affected, the strategy did improve the chronic fallout of cortical peritubular capillaries that occurred in the weeks after recovery from acute tubular necrosis; however, at the same time, the use of Ang-1* was associated with worsened fibrotic response, upregulated TGF-β expression, and increased renal inflammatory cells. Interestingly, the folic acid model differs from the obstructive nephropathy model in having a rise, rather than a decrease, in renal Ang-1 expression. Similarly, ischemic renal injury and angiotensin II infusion upregulate renal Ang-1 expression.

FUTURE PERSPECTIVES

Angiopoietin and Tie genes are expressed in the normal developing kidney, and the Tie genes are required for survival of metanephric capillaries. Tie-expressing endothelial precursors exist in the renal mesenchyme, and probably the same cells contribute to formation of glomerular capillaries. The roles of the angiopoietin and Tie genes in the mature, postnatal kidney remain uncertain. The effects of down-regulating endogenous, glomerular-derived Ang-1 have yet to be published, although, on the basis of the idea that Ang-2 can act as an Ang-1 antagonist, such animals may be found to share several features (e.g., glomerular endothelial apoptosis, albuminuria) of those that overexpress Ang-2 in glomeruli. The observation that Ang-2 is highly expressed in tubules that surround mature vasa rectae, which themselves express Tie-2, suggests that the factor has a special role in patterning and/or maintaining these vessels; however, Ang-2 null mice die neonatally, too soon to be informative for study of mature vasa rectae. Thus, proof of these ideas would require site-specific downregulation of Ang-2. Interestingly, nestin-expressing cells have been located around vasa rectae, and in certain experiments, can participate in angiogenesis, acquiring expression of Tie-2.

The observations that some epithelia express Tie genes should lead to a careful search for these receptors on subsets of renal epithelia, using confocal microscopy and imaging of cilia. If such expression could be established, then it may help to explain some nonendothelial effects observed when angiopoietins are overexpressed. Along the same lines, Ang-1 is implicated in branching morphogenesis of the developing lung, although whether this is a direct effect or is an indirect one mediated by enhanced angiogenesis is not known.

With regard to glomerular diseases, there is increasing evidence that upregulation of Ang-2 is a harmful event, destabilizing glomerular endothelia and perhaps having other, direct or indirect, actions on podocytes. Here, future studies could focus on upregulating Ang-1 expression locally, for example using Podocin-driven Ang-1 transgenic mice. Another possibility would be to sequestrate Ang-2 by expressing a soluble Tie-2 receptor within glomeruli; this strategy has been used in tumor and arthritis models associated with aberrant Ang/Tie signaling. Alternatively, Ang-2 could be specifically downregulated using RNA aptamers, as reported by White et al.

Ang-1 therapies show promise in the preservation of peritubular capillaries in chronic tubulointerstitial disease; however, such therapies may need to be tailored to specific primary kidney diseases in which endogenous Ang-1 levels are deficient; otherwise “too much of a good thing” may also cause enhanced fibrosis and inflammation. Along the same lines, we need to define whether different engineered forms of Ang-1 being used as therapies (Ang-1* versus COMP-Ang-1) have differential effects on blood endothelia versus nonendothelial cell biology.

Finally, there are limited data showing that Ang-3 is expressed in kidneys, for example by mesangial cells, and that total kidney levels are upregulated by hypoxia. In the future, studies should address the expression and potential roles of this factor and Ang-4 in renal disease.

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DISCLOSURES

None.

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