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REVIEW

Activin A: Autocrine Regulator of Kidney Development and Repair

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Abstract. The research described in this review suggests a novel and important role for activin A in the developmental and repair processes of the kidney (Table 1). The results obtained in these studies indicate that activin A is a negative regulator of kidney development and plays an essential part in kidney diseases, such as acute renal failure or renal fibrosis. It is also possible that activin A is a key player in the pathophysiological processes of other kidney diseases, such as congenital urogenital abnormalities, renal cystic disease and renal cell carcinoma. Activin A is thus a potential target for therapeutic interventions in kidney diseases. To address this issue, more detailed analysis on the regulation of activin production, modulation of activin activity and activin target genes is required.

Key words: Activin, Follistatin, Renal organogenesis, Kidney regeneration

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ACTIVINS are multifunctional cytokines belonging to the transforming growth factor (TGF)-β superfamily. Activins were originally discovered as inducers of follicle-stimulating hormone (FSH) release [1, 2], but are now known to regulate growth and differentiation in numerous biological systems, including erythropoiesis [3], neural cell survival [4] and early embryonic development [5–7]. Furthermore, in recent years, roles for activins have been identified in pancreatic β cell differentiation [8, 9], liver regeneration after hepatectomy [10, 11], angiogenesis [12, 13], systemic inflammation [14], branching morphogenesis of glandular organs [15] and tissue repair of various organs [16–18]. There is also evidence regarding the biological functions of activin A in the field of nephrology. In the present review, we will focus on a novel and important role of activin A in the developmental and regeneration processes of the kidney.

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Activin-Follistatin System

Activins are dimeric proteins consisting of β subunits, which are connected by disulfide bonds. There are three different forms of activin, homodimeric activin A (β_A-β_A) and activin B (β_B-β_B), as well as heteromeric activin AB (β_A-β_B). β_C [19], β_D [20] and β_E [21] chains have also been discovered, but their functions remain unknown. The activin signal is mediated by heteromeric receptor complexes consisting of two types of receptor, type I (ActR-I) and type II (ActR-II), which are characterized by an intracellular serine/threonine kinase domain [22] (Fig. 1). These receptors are structurally similar, with small cysteine-rich extracellular regions and intracellular regions consisting of kinase domains. ActR-I, but not ActR-II, has a region rich in glycine and serine residues (GS domain) in the juxtamembrane domain. Activin first binds with ActR-II, which is present in the cell membrane as an oligomeric form with an activated kinase. ActR-I, which also exists as an oligomeric form, but cannot bind to activin in the absence of ActR-II, is recruited to the complex. ActR-II then phosphorylates ActR-I in the GS domain, thereby activating it.

As an intracellular mediator of activin signaling,

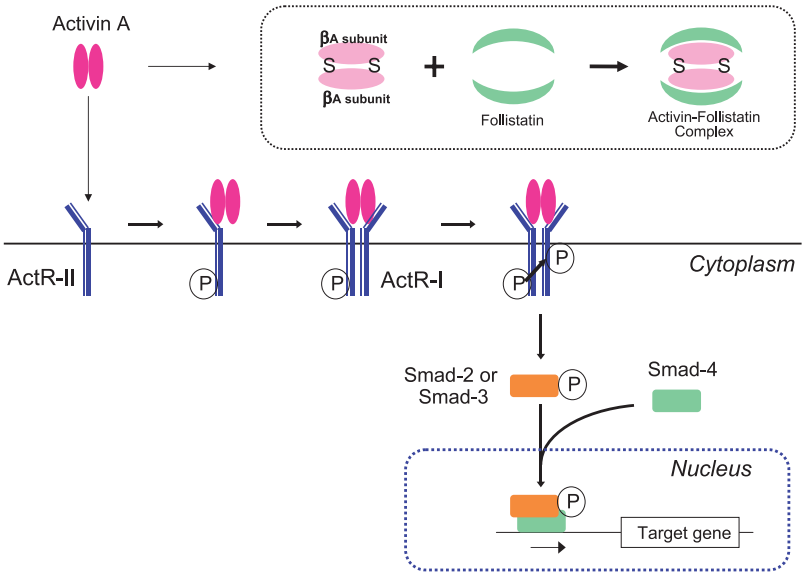

Fig. 1. Activin Signaling Pathway

Smad genes have been cloned. Of the eight Smads discovered, five (Smad-1, Smad-2, Smad-3, Smad-5, and Smad-8) are commonly referred to as receptor-regulated Smads (RSmads). Smad-1, Smad-5 and Smad-8 serve principally as substrates for bone morphogenetic protein (BMP) receptors, and Smad-2 and Smad-3 act as substrates for TGF-β, activin and nodal receptors. Smad-4, also referred as to Co-Smad, serves as a common partner for all RSmads. Smad-6 and Smad-7 are inhibitory Smads that serve as decoys to interfere with Smad-receptor or Smad-Smad interactions. Upon direct phosphorylation by ActR-I, Smad-2 or Smad-3 binds to its partner Smad-4 to form a heteromeric complex and translocates into the nucleus, where it can potentially regulate the transcription of target genes [23, 24] (Fig. 1). Recent studies have suggested additional Smad-independent signaling pathways for activin signaling, including Rho A, Mitogen-activated protein/ERK kinase kinase (MEKK)-1, c-Jun-NH2-terminal kinase (JNK) and p38 [25, 26].

The actions of activins are regulated by complex machinery at both the intracellular and extracellular levels. The most important modulator of the activin action is an activin-binding protein, follistatin. This protein binds to activins with high affinity and blocks their actions [27–29]. Follistatin is expressed on the surface of activin target cells by binding to the extracellular matrix [30]. Activins trapped by follistatin are then internalized by endocytosis and are subsequently degraded by proteolysis [31]. Several factors, such as α-2 macroglobin [32] and follistatin-related protein [33], also known as follistatin-related gene (FLRG) or follistatin-like 3 (FSTL-3), can bind to activin A. At the plasma membrane, Cripto inhibits activin/activin receptor interactions [34]. BAMBI (bone morphogenetic protein and activin membrane-bound inhibitor), a pseudoreceptor related to the type I receptors of the TGF-β family, inhibits activin A, TGF-β and BMP signaling by stably associating with TGF-β family receptors [35]. ARIPs (activin receptor-interacting proteins) regulate activin receptor type II activity and endocytosis [36]. In addition, several intracellular proteins and cofactors, including Smurf-type ubiquitin E3 ligases and Smad anchor for receptor activation [25], and transcriptional co-activators and co-repressors, such as CBP, p300, c-Ski and SnoN, control Smad activation by activin receptors, shuttling activated Smads into the nucleus and regulating transcriptional activity of Smad-containing complexes [24, 37].

Activin in Kidney Development

In mammals, the kidney develops in three stages: pronephros, mesonephros and metanephros. The metanephros then differentiates into the permanent kidney. Kidney organogenesis depends on a series of reciprocal inductive interactions between the epithelial

ureteric bud (UB) and metanephric mesenchyme (MM) [38]. Signals from the MM initiate kidney development by inducing formation of the UB from the Wolffian duct (WD). Subsequently, the growing UB also starts to branch in response to the mesenchymal signals and in return secretes signals that induce mesenchymal cells to condense and generate pretubular aggregates at the tips of UB branches. These aggregates undergo tubulogenesis via comma- and *S*-shaped bodies. The tubular structures finally fuse to form nephrons, the functional units of the kidney. Forming nephrons also attract endothelial cells to make functional glomeruli. However, it remains unclear how vascularization and innervation are regulated during kidney development. Using several approaches, such as *in vivo* studies with gene targeting techniques, *in vitro* organ culture systems using embryonic kidneys, and *in vitro* cell culture models, a large number of soluble factors, extracellular matrix, proteases and protease inhibitors involved in kidney development have been identified [39].

Inhibition of Ureteric Bud Outgrowth from the Wolffian Duct by Activin A

Development of the metanephric kidney begins with UB outgrowth from the WD. Ectopic budding of the UB from the WD leads to many congenital anomalies of the kidney and urinary tract, such as hypoplastic kidney, ectopia of the ureterovesical orifice, urinary outflow obstruction, and/or reflux [40, 41]. Elucidation of the budding mechanism is essential for understanding how these abnormalities develop. It is currently thought that UB emerges from the WD in response to glial-cell-derived neurotrophic growth factor (GDNF) produced by MM [42]. GDNF is a crucial positive signal in the budding process, but negative regulation of this process remains unclear.

A recent study has demonstrated that activin A negatively regulates bud formation in an *in vitro* WD culture system [43]. Immunohistochemical analysis demonstrated that activin A was expressed in the WD and phospho-Smad2/3, an intracellular mediator of activin signaling, was detectable in the nuclei of the WD cells, suggesting that the activin signaling pathway is normally active along the WD. When cultured with the surrounding mesonephros, WDs formed numerous ectopic buds in response to GDNF. Activin A completely inhibited GDNF-induced bud formation,

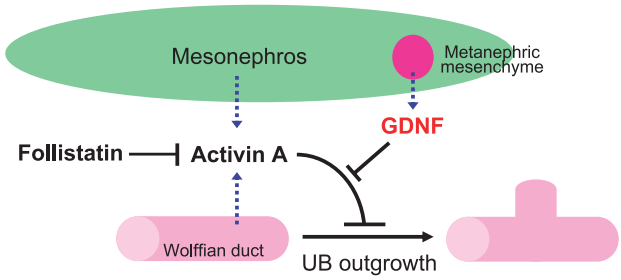

Fig. 2. Negative Regulation of UB Outgrowth from WD by Activin A

which was accompanied by inhibition of cell proliferation, reduced expression of Pax-2, and decreased phospholyration of PI3 kinase and MAP kinase in the WD. To further clarify the role of activin A, the effects of activin blockade on WD cultured in the absence of mesonephros were also tested. WDs without mesonephros did not form ectopic buds, even in the presence of GDNF. Interestingly, blockade of activin action with a variety of agents (natural antagonist, neutralizing antibodies, siRNA) enabled GDNF to induce ectopic buds. In the ectopic buds induced by GDNF, activin A was absent and phospho-Smad2/3 was undetectable. These data suggest that activin A is an endogenous inhibitor of bud formation and that cancellation of activin A action may be critical for the initiation of this process (Fig. 2) [43].

Delayed Branching of Ureteric Bud by Activin A

Branching morphogenesis of the UB is positively and negatively regulated by various factors [39]. It has been demonstrated that activin A plays a critical role in this process [44, 45]. Activin A is produced by the UB, and activin type II receptors are localized in UB and MM. In an organ culture system, exogenous activin A reduced the size of cultured metanephroi, delayed UB branching, and enlarged UB tips. Exogenous follistatin enlarged the size of cultured metanephroi, increased UB branching, and promoted cell growth in the UB. Blockade of activin signaling by adenoviral transfection of dominantly negative activin mutant receptor mimics the effect of follistatin. Similar results have been obtained in an isolated UB culture system [46]. Taken together, it is possible that activin A produced by UB is an autocrine factor that negatively regulates UB branching. The effect of activin A on MM has also been analyzed. In cultured metanephric mes-

enchymal cells, activin A promoted cell growth and conversely, follistatin induced apoptosis. Furthermore, activin A induced the expression of epithelial differentiation markers in these cells, suggesting that activin A not only regulates UB branching but is also required for MM differentiation during kidney development [45].

Phenotype of Activins or Activin Receptors Knockout Mice

In order to analyze the physiological role of activins, various mutant mice lacking genes encoding activin subunits and receptors were developed by homologous recombination [47]. However, none of these mice developed any kidney abnormalities. Given that activins have been demonstrated to play a role in kidney development *in vitro*, the abnormalities found in these mutant mice were less than expected. This may be due to the existence of multiple activin subunits, and redundancy in the activin signaling system.

As an alternative approach to study the physiological role of activins, transgenic mice expressing the truncated type II activin receptor (tActR-II), which acts as a dominant-negative receptor and blocks the action of activins and related ligands, were developed [48]. In these mice, the gross morphological appearance of the kidneys was normal, and the size and wet weight of the kidneys were identical to those of normal mice. Serum concentrations of blood urea nitrogen (BUN) and creatinine, as well as creatinine clearance, were also identical to those in normal mice. However, histological examination revealed an increased number of glomeruli in the kidneys of the transgenic mice. Morphometrically, the number of glomeruli in transgenic mouse kidneys was approximately 180% that of normal mice. The mean area of a single glomerulus was significantly smaller than that in normal mice. Morphologically, there were no apparent abnormalities in glomerular components, including mesangial cells, matrix, endothelial cells and basement membrane. Branching of the UB determines the number of nephrons. Given that activin A inhibits the branching morphogenesis of the UB in organ culture systems [44–46], blockade of activin action would probably enhance the branching of the UB and thereby increase the number of nephrons. The action of activin or related ligands might be critical in the determination of nephron number.

Modulation of MDCK Tubulogenesis by the Activin-Follistatin System

MDCK cells cultured in collagen gels form branching tubules that exhibit apical-basolateral polarity in the presence of fibroblast-conditioned medium [49] or HGF [50]. This *in vitro* kidney tubulogenesis model provides an opportunity to test the effects of growth factors, which seem to be involved in kidney development, on tube formation without interaction with other cell types. Using this system, the role of the activin-follistatin system in branching tubulogenesis has been investigated [51]. MDCK cells expressed mRNA for the β_A subunit of activin as well as follistatin. These cells formed spherical cysts when cultured in collagen gel, and HGF added to these spherical cysts induced branching tubulogenesis. When activin A was added together with HGF, the branching tubulogenesis induced by HGF was blocked in a dose-dependent manner and the activin-treated cells were scattered. Conversely, follistatin induced branching tubulogenesis in the absence of HGF. Thin section electron microscopy showed polarized tubular formation with smooth basal surface in contact with the collagen gel and microvilli-rich apical surface facing the lumen. Interestingly, adenoviral transfection of the truncated activin type II receptor (AdextARII), which lacks the intracellular serine/threonine kinase domain, also induced branching tubulogenesis. HGF markedly reduced the expression of the β_A subunit in MDCK cells cultured in collagen gel. The level of mRNA expression for the β_A subunit of activin in branching tubules was markedly lower than that in cysts. These data suggest that activin A produced by MDCK cells inhibits branching tubulogenesis and HGF-induced branching tubulogenesis by blocking the production of activin.

Activin in Kidney Disease

Tubular cells have the potential to regenerate following variety of insults. During acute tubular necrosis induced by renal ischemia or renal toxins, normal quiescent cells undergo dedifferentiation and regain their potential to divide after enhancing DNA synthesis. Following proliferation, the new cells then differentiate to restore the functional integrity of nephrons [52, 53]. Recent evidence suggests that growth factors critical for kidney development are potentially in-

volved in the repair process of the kidney and play important roles in these processes as mitogens, motogens and morphogens [39]. Activation of the regeneration process with these renotropic factors is considered a new approach for the treatment of kidney disease. The specific factors that play a critical role in kidney regeneration are of great interest.

Regulation of Repair Process of Injured Tubules by Activin-Follistatin System

The activin-follistatin system plays an important role in kidney development. However, its biological effects in this organ after completion of development are unknown. The role of the activin-follistatin system in tissue repair and regeneration processes of the kidney has been extensively examined using an ischemia/reperfusion injury model [54]. As in other tissues, the expression of activin and follistatin changed significantly in the kidneys after ischemic injury. No expression of mRNA for the activin β_A subunit was detected in normal adult kidneys, but its expression increased markedly after renal ischemia. In addition, mRNA expression of the activin receptors was detected in normal kidneys, but was not altered after renal ischemia. Follistatin is expressed abundantly in adult kidneys. Intense localization of follistatin protein was observed in the cuboidal epithelial lining of distal and collecting tubules, as well as proximal tubules [55]. *In situ* hybridization revealed that the expression of mRNA for follistatin, abundant in tubular cells of the outer medulla of normal kidneys, decreased significantly after renal ischemia. To assess the role of endogenous activin A in renal regeneration, recombinant human follistatin was infused intravenously into rats with renal ischemia at the time of reperfusion. In control (saline-treated) rats, renal tubules were dilated and hemorrhagic changes were observed. In follistatin-treated rats, these abnormalities were markedly reduced. Similarly, apoptotic changes in renal tubules, as assessed by TUNEL method, were prevented by follistatin. Furthermore, proliferation of tubule cells, as assessed by bromodeoxyuridine labeling, was greatly increased in follistatin-treated rats. As expected, serum levels of creatinine and blood urea nitrogen were also significantly lower in follistatin-treated rats. These results support the notion that elevated production of activin A enhances tubular damage and inhibits the regeneration processes of damaged tubules. Consistent with this notion, intravenous administration of recombinant activin A inhibited tubular cell proliferation after ischemic injury. Follistatin administered intravenously accelerates renal regeneration following renal ischemia by blocking the action of endogenous activin, thus indicating that the blockade of endogenous growth inhibitors is an alternative method for accelerating renal regeneration, instead of growth promoting factors infusion.

The repair processes in many tissues have long been thought to have similarities to developmental processes. For example, vimentin is an intermediate filament and a marker of undifferentiated mesenchymal cells. It is not present in normal tubular cells, but its expression was observed during tubular regeneration [56]. During tissue regeneration, a cascade of developmental gene pathways is apparently reactivated. The transcription factor Pax-2 is thought to play a key regulatory role during renal organogenesis [57]. Pax-2 deficiency causes defective growth of the fetal kidney and ureter [58], while overexpression is associated with epithelial overgrowth, including cysts or tumor formation [59, 60]. It was recently shown that Pax-2, which is not observed in differentiated tubular epithelia, is present in tubular cells after renal injury induced by a renal toxin [61]. Furthermore, reactivation of Pax-2 was observed in the nuclei of regenerating tubular cells after ischemic injury [62]. The number of Pax-2-positive cells in ischemic kidneys increased after administration of follistatin and, conversely, reduced after administration of activin A. In a renal tubular epithelial cell line, the inactivation of activin signaling pathways induced the upregulation of Pax-2 expression [63]. These results indicate that the activin-follistatin system modulates renal regeneration after injury by controlling the proliferation of Pax-2-positive cells [62].

Activin and Renal Fibrosis

Like TGF-β, activins have been shown to be important regulators in tissue fibrosis in various organs [16]. The role of activin A in the fibrotic process of the kidney was recently analyzed using a unilateral ureter obstruction (UUO) model [64]. Immunoreactive activin A was upregulated in tubular cells in the kidneys with UUO, but not in normal and contralateral kidneys. Activin A promoted cell proliferation, enhanced the expression of type I collagen mRNA, and induced the

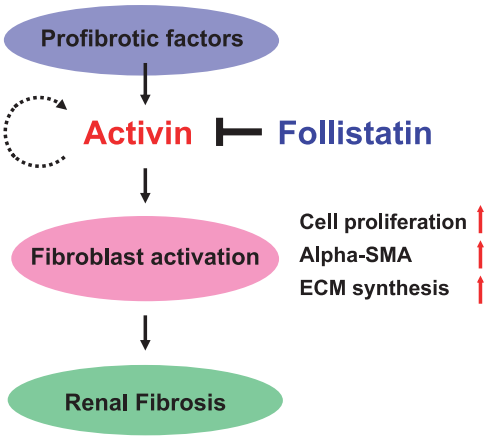

Fig. 3. Possible Mechanism of Activin Action in Renal Fibrosis

production of α-smooth muscle actin [65] in a rat kidney fibroblast cell line (NRK-49F cells) as well as in primary cultured renal interstitial fibroblasts. In contrast, activin A did not affect the expression of α-SMA and type I collagen in renal epithelial tubular cell lines LLC-PK1 and MDCK. Follistatin significantly inhibited cell proliferation in NRK-49F cells. The expression of activin A was induced by TGF-β1 or activin A itself. Induction of type I collagen expression by TGF-β1 was reduced by follistatin or by overexpression of truncated type II activin receptor. These results suggest that activin A produced by tubular cells activates renal interstitial fibroblasts in a paracrine fashion during the fibrotic processes of the kidney (Fig. 3).

A recent study has suggested a role for activin A in glomerulonephritis [66]. In cultured mesangial cells, interleukin-1 (IL-1) and basic fibroblast growth factor (bFGF), both mediators of glomerular inflammatory injury, dose-dependently increased activin A expression. Incubation with activin A significantly stimulated TGF-β1, plasminogen activator inhibitor [67]-1, and connective tissue growth factor (CTGF) expression and increased production of extracellular matrix proteins in mesangial cells. In a rat model of glomerular fibrosis (anti-Thy1 glomerulonephritis), there was a transient increase in the expression of glomerular activin A mRNA and protein, which was paralleled with expression of TGF-β. These data suggest that activin A is a pro-fibrotic cytokine and modulates glomerular matrix expansion during the development of glomerulonephritis.

Table 1. Functions of Activin in Developmental, Regenerative and Fibrotic Processes of Kidney

1) Kidney organogenesis
   a. *Isolated Wolffian duct culture* [43]
      – Activin A inhibits ureteric bud outgrowth from the Wolffian duct
   b. *Metanephric kidney culture* [44, 45, 46]
      – Activin A inhibits ureteric bud branching
   c. *Transgenic mice expressing truncated activin mutant receptor* [48]
      – Number of glomeruli was increased in transgenic mice when compared to wild-type mice
   d. *In vitro tubulogenesis model* [51]
      – Blockade of activin action induced branching tubulogenesis

2) Tubular regeneration
   a. *Ischemia-reperfusion injury model* [54, 62]
      – Activin A was upregulated in kidney after renal ischemia
      – Exogenous follistatin enhanced tubular regeneration after renal ischemia and increased the number of Pax-2-positive cells
   b. *Renal tubular epithelial cell line* [63]
      – Activin A inhibited cell proliferation and induced cell differentiation

3) Renal fibrosis
   a. *Unilateral Ureteral Obstruction model* [64]
      – Activin A was upregulated in tubular cells after UUO
   b. *Primary renal interstitial fibroblasts* [64]
      – Activin A promoted cell proliferation, induced alpha-SMA expression, and increased production of type I collagen
   c. *Primary rat mesangial cells* [65]
      – Activin increased production of extracellular matrix proteins

References are indicated in parentheses.

References

1. Ling N, Ying SY, Ueno N, Shimasaki S, Esch F, Hotta M, Guillemin R (1986) Pituitary FSH is released by a heterodimer of the beta-subunits from the two forms of inhibin. *Nature* 321: 779–782.
2. Vale W, Rivier J, Vaughan J, McClintock R, Corrigan A, Woo W, Karr D, Spiess J (1986) Purification and characterization of an FSH releasing protein from porcine ovarian follicular fluid. *Nature* 321: 776–779.

3. Murata M, Eto Y, Shibai H, Sakai M, Muramatsu M (1988) Erythroid differentiation factor is encoded by the same mRNA as that of the inhibin beta A chain. *Proc Natl Acad Sci USA* 85: 2434–2438.
4. Schubert D, Kimura H, LaCorbiere M, Vaughan J, Karr D, Fischer WH (1990) Activin is a nerve cell survival molecule. *Nature* 344: 868–870.
5. Smith JC, Price BM, Van Nimmen K, Huylebroeck D (1990) Identification of a potent Xenopus mesoderm-inducing factor as a homologue of activin A. *Nature* 345: 729–731.
6. van den Eijnden-Van Raaij AJ, van Zoelent EJ, van Nimmen K, Koster CH, Snoek GT, Durston AJ, Huylebroeck D (1990) Activin-like factor from a Xenopus laevis cell line responsible for mesoderm induction. *Nature* 345: 732–734.
7. Thomsen G, Woolf T, Whitman M, Sokol S, Vaughan J, Vale W, Melton DA (1990) Activins are expressed early in Xenopus embryogenesis and can induce axial mesoderm and anterior structures. *Cell* 63: 485–493.
8. Mashima H, Ohnishi H, Wakabayashi K, Mine T, Miyagawa J, Hanafusa T, Seno M, Yamada H, Kojima I (1996) Betacellulin and activin A coordinately convert amylase-secreting pancreatic AR42J cells into insulin-secreting cells. *J Clin Invest* 97: 1647–1654.
9. Zhang YQ, Zhang H, Maeshima A, Kurihara H, Miyagawa J, Takeuchi T, Kojima I (2002) Up-regulation of the expression of activins in the pancreatic duct by reduction of the beta-cell mass. *Endocrinology* 143: 3540–3547.
10. Kogure K, Omata W, Kanzaki M, Zhang YQ, Yasuda H, Mine T, Kojima I (1995) A single intraportal administration of follistatin accelerates liver regeneration in partially hepatectomized rats. *Gastroenterology* 108: 1136–1142.
11. Kogure K, Zhang YQ, Maeshima A, Suzuki K, Kuwano H, Kojima I (2000) The role of activin and transforming growth factor-beta in the regulation of organ mass in the rat liver. *Hepatology* 31: 916–921.
12. Maeshima K, Maeshima A, Hayashi Y, Kishi S, Kojima I (2004) Crucial role of activin a in tubulogenesis of endothelial cells induced by vascular endothelial growth factor. *Endocrinology* 145: 3739–3745.
13. Poulaki V, Mitsiades N, Kruse FE, Radetzky S, Iliaki E, Kirchhof B, Joussen AM (2004) Activin a in the regulation of corneal neovascularization and vascular endothelial growth factor expression. *Am J Pathol* 164: 1293–1302.
14. Jones KL, de Kretser DM, Patella S, Phillips DJ (2004) Activin A and follistatin in systemic inflammation. *Mol Cell Endocrinol* 225: 119–125.
15. Ball EM, Risbridger GP (2001) Activins as regulators of branching morphogenesis. *Dev Biol* 238: 1–12.
16. Werner S, Alzheimer C (2006) Roles of activin in tissue repair, fibrosis, and inflammatory disease. *Cytokine Growth Factor Rev* 17: 157–171.
17. Maeshima A, Nojima Y, Kojima I (2001) The role of the activin-follistatin system in the developmental and regeneration processes of the kidney. *Cytokine Growth Factor Rev* 12: 289–298.
18. Kojima I, Maeshima A, Zhang YQ (2001) Role of the activin-follistatin system in the morphogenesis and regeneration of the renal tubules. *Mol Cell Endocrinol* 180: 179–182.
19. G Ht, Neidhardt H, Schneider C, Pohl J (1995) Cloning of a new member of the TGF-beta family: a putative new activin beta C chain. *Biochem Biophys Res Commun* 206: 608–613.
20. Oda S, Nishimatsu S, Murakami K, Ueno N (1995) Molecular cloning and functional analysis of a new activin beta subunit: a dorsal mesoderm-inducing activity in Xenopus. *Biochem Biophys Res Commun* 210: 581–588.
21. Fang J, Yin W, Smiley E, Wang SQ, Bonadio J (1996) Molecular cloning of the mouse activin beta E subunit gene. *Biochem Biophys Res Commun* 228: 669–674.
22. Welt C, Sidis Y, Keutmann H, Schneyer A (2002) Activins, inhibins, and follistatins: from endocrinology to signaling. A paradigm for the new millennium. *Exp Biol Med (Maywood)* 227: 724–752.
23. ten Dijke P, Hill CS (2004) New insights into TGF-beta-Smad signalling. *Trends Biochem Sci* 29: 265–273.
24. Shi Y, Massague J (2003) Mechanisms of TGF-beta signaling from cell membrane to the nucleus. *Cell* 113: 685–700.
25. Zhang L, Deng M, Parthasarathy R, Wang L, Mongan M, Molkentin JD, Zheng Y, Xia Y (2005) MEKK1 transduces activin signals in keratinocytes to induce actin stress fiber formation and migration. *Mol Cell Biol* 25: 60–65.
26. Cocolakis E, Lemay S, Ali S, Lebrun JJ (2001) The p38 MAPK pathway is required for cell growth inhibition of human breast cancer cells in response to activin. *J Biol Chem* 276: 18430–18436.
27. Ueno N, Ling N, Ying SY, Esch F, Shimasaki S, Guillemin R (1987) Isolation and partial characterization of follistatin: a single-chain Mr 35,000 monomeric protein that inhibits the release of follicle-stimulating hormone. *Proc Natl Acad Sci USA* 84: 8282–8286.
28. Shimonaka M, Inouye S, Shimasaki S, Ling N (1991) Follistatin binds to both activin and inhibin through the common subunit. *Endocrinology* 128: 3313–3315.
29. Nakamura T, Takio K, Eto Y, Shibai H, Titani K, Sugino H (1990) Activin-binding protein from rat ovary is follistatin. *Science* 247: 836–838.
30. Nakamura T, Sugino K, Titani K, Sugino H (1991) Follistatin, an activin-binding protein, associates with heparan sulfate chains of proteoglycans on follicular granulosa cells. *J Biol Chem* 266: 19432–19437.

31. Hashimoto O, Nakamura T, Shoji H, Shimasaki S, Hayashi Y, Sugino H (1997) A novel role of follistatin, an activin-binding protein, in the inhibition of activin action in rat pituitary cells. Endocytotic degradation of activin and its acceleration by follistatin associated with cell-surface heparan sulfate. *J Biol Chem* 272: 13835–13842.
32. Mather JP (1996) Follistatins and alpha 2-macroglobulin are soluble binding proteins for inhibin and activin. *Horm Res* 45: 207–210.
33. Schneyer A, Tortoriello D, Sidis Y, Keutmann H, Matsuzaki T, Holmes W (2001) Follistatin-related protein (FSRP): a new member of the follistatin gene family. *Mol Cell Endocrinol* 180: 33–38.
34. Adkins HB, Bianco C, Schiffer SG, Rayhorn P, Zafari M, Cheung AE, Orozco O, Olson D, De Luca A, Chen LL, Miatkowski K, Benjamin C, Normanno N, Williams KP, Jarpe M, LePage D, Salomon D, Sanicola M (2003) Antibody blockade of the Cripto CFC domain suppresses tumor cell growth *in vivo*. *J Clin Invest* 112: 575–587.
35. Onichtchouk D, Chen YG, Dosch R, Gawantka V, Delius H, Massague J, Niehrs C (1999) Silencing of TGF-beta signalling by the pseudoreceptor BAMBI. *Nature* 401: 480–485.
36. Tsuchida K, Nakatani M, Matsuzaki T, Yamakawa N, Liu Z, Bao Y, Arai KY, Murakami T, Takehara Y, Kurisaki A, Sugino H (2004) Novel factors in regulation of activin signaling. *Mol Cell Endocrinol* 225: 1–8.
37. Chen YG, Wang Q, Lin SL, Chang CD, Chuang J, Ying SY (2006) Activin signaling and its role in regulation of cell proliferation, apoptosis, and carcinogenesis. *Exp Biol Med (Maywood)* 231: 534–544.
38. Saxén L: *Organogenesis of the kidney,* Cambridge; New York, Cambridge University PRess 1987.
39. Shah MM, Sampogna RV, Sakurai H, Bush KT, Nigam SK (2004) Branching morphogenesis and kidney disease. *Development* 131: 1449–1462.
40. Ichikawa I, Kuwayama F, Pope JCt, Stephens FD, Miyazaki Y (2002) Paradigm shift from classic anatomic theories to contemporary cell biological views of CAKUT. *Kidney Int* 61: 889–898.
41. Pohl M, Bhatnagar V, Mendoza SA, Nigam SK (2002) Toward an etiological classification of developmental disorders of the kidney and upper urinary tract. *Kidney Int* 61: 10–19.
42. Shakya R, Watanabe T, Costantini F (2005) The role of GDNF/Ret signaling in ureteric bud cell fate and branching morphogenesis. *Dev Cell* 8: 65–74.
43. Maeshima A, Vaughn DA, Choi Y, Nigam SK (2006) Activin A is an endogenous inhibitor of ureteric bud outgrowth from the Wolffian duct. *Dev Biol* 295: 473–485.
44. Ritvos O, Tuuri T, Eramaa M, Sainio K, Hilden K, Saxen L, Gilbert SF (1995) Activin disrupts epithelial branching morphogenesis in developing glandular organs of the mouse. *Mech Dev* 50: 229–245.
45. Maeshima A, Yamashita S, Maeshima K, Kojima I, Nojima Y (2003) Activin a produced by ureteric bud is a differentiation factor for metanephric mesenchyme. *J Am Soc Nephrol* 14: 1523–1534.
46. Bush KT, Sakurai H, Steer DL, Leonard MO, Sampogna RV, Meyer TN, Schwesinger C, Qiao J, Nigam SK (2004) TGF-beta superfamily members modulate growth, branching, shaping, and patterning of the ureteric bud. *Dev Biol* 266: 285–298.
47. Chang H, Brown CW, Matzuk MM (2002) Genetic analysis of the mammalian transforming growth factor-beta superfamily. *Endocr Rev* 23: 787–823.
48. Maeshima A, Shiozaki S, Tajima T, Nakazato Y, Naruse T, Kojima I (2000) Number of glomeruli is increased in the kidney of transgenic mice expressing the truncated type II activin receptor. *Biochem Biophys Res Commun* 268: 445–449.
49. Montesano R, Schaller G, Orci L (1991) Induction of epithelial tubular morphogenesis *in vitro* by fibroblast-derived soluble factors. *Cell* 66: 697–711.
50. Montesano R, Matsumoto K, Nakamura T, Orci L (1991) Identification of a fibroblast-derived epithelial morphogen as hepatocyte growth factor. *Cell* 67: 901–908.
51. Maeshima A, Zhang YQ, Furukawa M, Naruse T, Kojima I (2000) Hepatocyte growth factor induces branching tubulogenesis in MDCK cells by modulating the activin-follistatin system. *Kidney Int* 58: 1511–1522.
52. Bonventre JV, Weinberg JM (2003) Recent advances in the pathophysiology of ischemic acute renal failure. *J Am Soc Nephrol* 14: 2199–2210.
53. Devarajan P (2006) Update on mechanisms of ischemic acute kidney injury. *J Am Soc Nephrol* 17: 1503–1520.
54. Maeshima A, Zhang YQ, Nojima Y, Naruse T, Kojima I (2001) Involvement of the activin-follistatin system in tubular regeneration after renal ischemia in rats. *J Am Soc Nephrol* 12: 1685–1695.
55. Kogawa K, Ogawa K, Hayashi Y, Nakamura T, Titani K, Sugino H (1991) Immunohistochemical localization of follistatin in rat tissues. *Endocrinol Jpn* 38: 383–391.
56. Witzgall R, Brown D, Schwarz C, Bonventre JV (1994) Localization of proliferating cell nuclear antigen, vimentin, c-Fos, and clusterin in the postischemic kidney. Evidence for a heterogenous genetic response among nephron segments, and a large pool of mitotically active and dedifferentiated cells. *J Clin Invest* 93: 2175–2188.
57. Dressler GR (2006) The cellular basis of kidney development. *Annu Rev Cell Dev Biol* 22: 509–529.
58. Torres M, Gomez-Pardo E, Dressler GR, Gruss P (1995) Pax-2 controls multiple steps of urogenital de-

velopment. *Development* 121: 4057–4065.
59. Dressler GR, Wilkinson JE, Rothenpieler UW, Patterson LT, Williams-Simons L, Westphal H (1993) Deregulation of Pax-2 expression in transgenic mice generates severe kidney abnormalities. *Nature* 362: 65–67.
60. Winyard PJ, Risdon RA, Sams VR, Dressler GR, Woolf AS (1996) The PAX2 tanscription factor is expressed in cystic and hyperproliferative dysplastic epithelia in human kidney malformations. *J Clin Invest* 98: 451–459.
61. Imgrund M, Grone E, Grone HJ, Kretzler M, Holzman L, Schlondorff D, Rothenpieler UW (1999) Re-expression of the developmental gene Pax-2 during experimental acute tubular necrosis in mice 1. *Kidney Int* 56: 1423–1431.
62. Maeshima A, Maeshima K, Nojima Y, Kojima I (2002) Involvement of Pax-2 in the action of activin A on tubular cell regeneration. *J Am Soc Nephrol* 13: 2850–2859.
63. Maeshima A, Nojima Y, Kojima I (2002) Activin A: an autocrine regulator of cell growth and differentiation in renal proximal tubular cells. *Kidney Int* 62: 446–454.
64. Yamashita S, Maeshima A, Kojima I, Nojima Y (2004) Activin A is a potent activator of renal interstitial fibroblasts. *J Am Soc Nephrol* 15: 91–101.
65. Wagers AJ, Sherwood RI, Christensen JL, Weissman IL (2002) Little evidence for developmental plasticity of adult hematopoietic stem cells. *Science* 297: 2256–2259.
66. Gaedeke J, Boehler T, Budde K, Neumayer HH, Peters H (2005) Glomerular activin A overexpression is linked to fibrosis in anti-Thy1 glomerulonephritis. *Nephrol Dial Transplant* 20: 319–328.
67. Tumbar T, Guasch G, Greco V, Blanpain C, Lowry WE, Rendl M, Fuchs E (2004) Defining the epithelial stem cell niche in skin. *Science* 303: 359–363.