pharynx. On E17.5, strong expression of USAG-1 mRNA was confined to the kidney tubules and ameloblasts of teeth. In addition, moderate expression was observed in hair follicles, choroids plexus, and ependymal cells in the ventricles of the brain.

In adult tissues, the expression was by far most abundant in the kidney and is restricted to the distal tubules. No expression was observed in proximal tubules, glomeruli, or blood vessels in the kidney. Thus, the cellular distribution of USAG-1 is overlapping with that of BMP-7 in the kidney. Taken together with the fact that proximal tubule epithelial cells (PTECs) are the site of injuries in many types of kidney diseases, and that PTECs express the receptors for BMP-7, we hypothesized the working model about the regulation of renoprotective action of BMP-7 (Figure 1a): in renal injuries, PTECs are mainly damaged and undertake apoptosis or EMT to fibroblast-like mesenchymal cells. BMP-7 secreted from distal tubules binds to the receptors in the cell surface of PTECs, and inhibits apoptosis and EMT. USAG-1 is also secreted from distal tubules, binds to BMP-7, and inhibits the renoprotective actions of BMP-7 by reducing the amount of available BMP-7.

To evaluate this working model, our group generated *USAG-1* knockout (*USAG-1*$^{-/-}$) mice, and induced acute

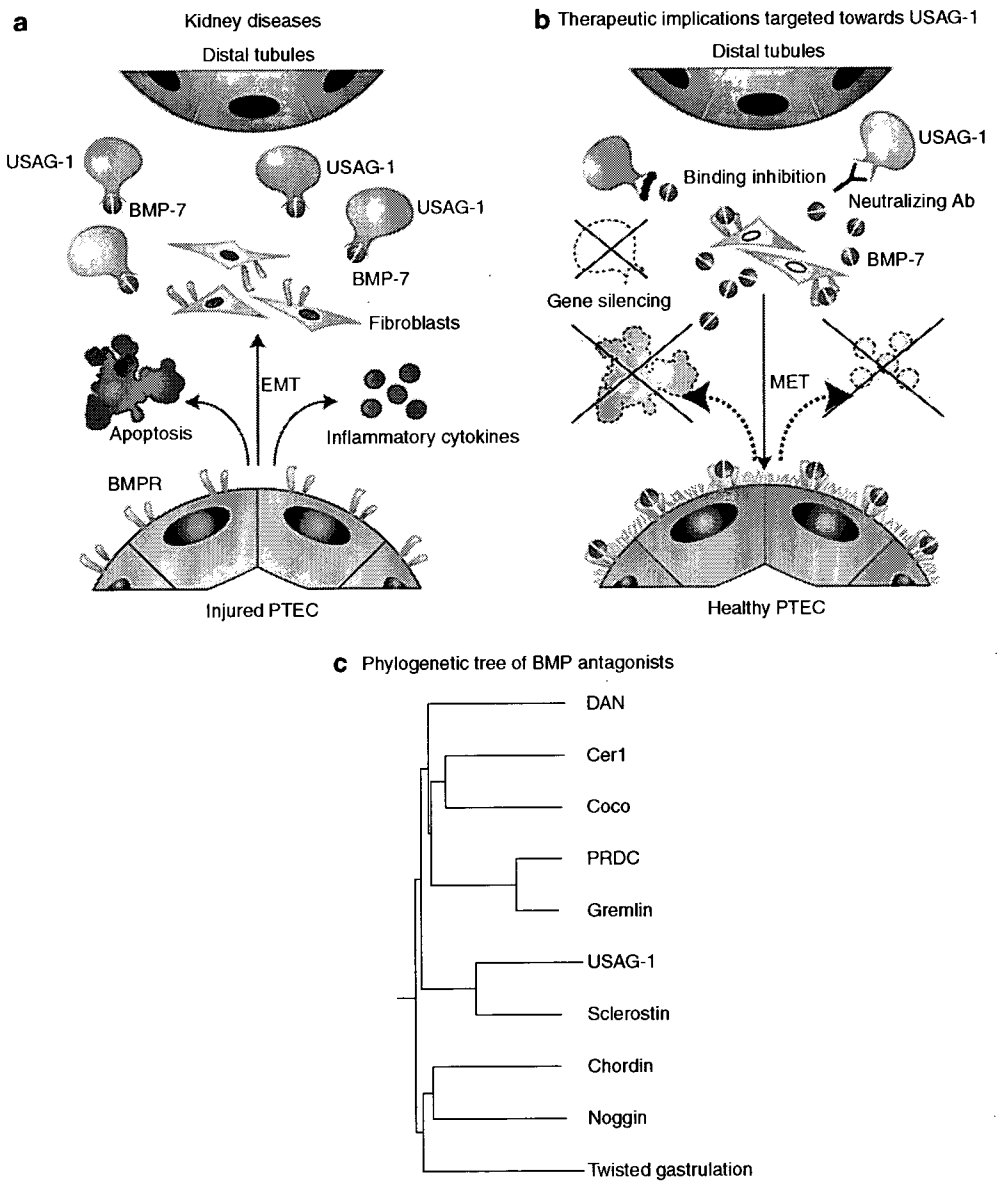

Figure 1 | Working hypothesis. (a) In kidney diseases, injured PTECs undertake apoptosis and EMT, and produce inflammatory cytokines. BMP-7 secreted from distal tubules is known to inhibit apoptosis, EMT, and production of cytokines of PTEC. USAG-1 is secreted from distal tubules, binds to BMP-7, and inhibits the binding of BMP-7 to its receptors. **(b)** Drugs or neutralizing antibodies that inhibits binding between USAG-1 and BMP, or gene-silencing therapy for USAG-1 would increase available endogenous BMP, and might be a promising way to develop novel therapeutic methods for severe renal diseases. **(c)** Phylogenetic tree of BMP antagonists. Phylogenetic tree of human BMP antagonists based on the overall amino-acid sequence similarity. GenomeNet server at http://www.genome.jp/ was used for phylogenetic tree construction.

and chronic renal disease models in which the renal tubules were mainly damaged.

USAG-1$^{-/-}$ mice are resistant to kidney tubular injury

USAG-1$^{-/-}$ mice were born at the ratio expected by Mendel's law of heredity, and were viable, fertile, and appeared healthy, except that *USAG-1*$^{-/-}$ mice exhibit supernumerary teeth, both in the incisors and molars, and fused teeth in the molar teeth region.[14] Although there was variation in the sites of extra teeth and fused teeth, these teeth phenotype was fully penetrant.

Because the renal function and histology of the kidney in *USAG-1*$^{-/-}$ mice appears normal, our group challenged the mice with two different kidney disease models and found that *USAG-1*$^{-/-}$ mice are resistant to renal injury.

As a model for acute renal failure, we utilized cisplatin nephrotoxicity model.[14] Administration of cisplatin to wild-type (WT) littermates causes acute tubular injuries that result in severe renal failure. Within the first 3 days, 54% of WT mice died, whereas 92% of *USAG-1*$^{-/-}$ mice survived the period. Renal function and histology of *USAG-1*$^{-/-}$ mice at day 3 was significantly preserved when compared to WT littermate. Tubular apoptosis, a characteristic feature of cisplatin nephrotoxicity, was also significantly reduced in *USAG-1*$^{-/-}$ mice.

As a model of chronic renal injury, unilateral ureteral obstruction was performed in both *USAG-1*$^{-/-}$ mice and WT mice, and the kidneys were harvested 14 days after the operation. In WT mice, the obstructed kidney showed degeneration of renal tubules and interstitial fibrosis, whereas normal architecture was preserved in *USAG-1*$^{-/-}$ mice, except for mild dilatation of tubules. Expression of E-cadherin, a marker for tubular epithelial integrity, was severely reduced in the kidney of WT mice, whereas its expression was preserved in *USAG-1*$^{-/-}$ mice.

Renal BMP signaling, assessed by phosphorylation of Smad proteins, is significantly enhanced in *USAG-1*$^{-/-}$ mice during renal injury, indicating that the preservation of renal function might be attributable to enhancement of endogenous BMP signaling.

Furthermore, the administration of neutralizing antibody against BMP-7 abolished renoprotection in *USAG-1*$^{-/-}$ mice. These results strongly support the working model, and BMP-7 is the potent candidate for the counterpart of USAG-1.

Interestingly, the expression of USAG-1 decreases during the course of disease models. Reduction of USAG-1 in kidney diseases might be a kind of self-defense mechanism to minimize the inhibitory effect on BMP signaling. Because the reduction of USAG-1 expression in WT mice is not enough to overcome the reduction of BMP-7 expression, further reduction or abolishment of the action of USAG-1 is desirable for the preservation of renal function, and the results in the present study justify the therapy targeted toward USAG-1. For example, drugs or neutralizing antibodies that inhibits binding between USAG-1 and BMP, or gene-silencing therapy for USAG-1 would enhance the activities of endogenous BMP, and might be a promising way to develop novel therapeutic methods for severe renal diseases (Figure 1b). Because the expression of USAG-1 is confined to the kidney in adult mice and humans, it would be a better target for kidney-specific therapeutic trials. On the contrary, the administration of recombinant BMP-7 protein, whose target cells are widely distributed throughout the body, might produce some additional extra-renal actions, which includes beneficial effects, such as actions on renal osteodystrophy and vascular calcification. Furthermore, these therapy targeted toward USAG-1 might protect the kidney during the administration of nephrotoxic agents such as cisplatin.

However, because most of the causes of end-stage renal diseases are glomerular origin, pathological roles of USAG-1 in glomerular injuries should be elucidated before undertaking therapeutic trials against USAG-1. In addition, elucidation of physiological and developmental function of USAG-1 is also essential.

USAG-1 is the most abundant BMP antagonist in adult kidney

Our group demonstrated that USAG-1 is by far the most abundant BMP antagonist in the kidney.[14] The expression of USAG-1 and other BMP antagonists in adult kidneys were compared by modified real-time polymerase chain reaction with the standard curve using various concentrations of plasmid encoding each BMP antagonist, and the copy number of each genes in kidney cDNA were determined.

As a result, USAG-1 was by far the most abundant in the kidneys among known BMP antagonists. Because other BMP antagonists also antagonize BMP-7 activities, it is concluded that USAG-1 plays important role in the modulation of BMP activities in the kidney not because of its ligand specificity, but because of its high expression among other BMP antagonists. In addition, localization of USAG-1 is quite similar to that of BMP-7, so that USAG-1 can effectively access to and inactivate BMP-7 at the site of production.

USAG-1 in teeth development

USAG-1 is also expressed in developing teeth, and a USAG-1-positive area surrounds the enamel knot signaling centers where BMPs are expressed.[16] As mentioned earlier, *USAG-1*$^{-/-}$ mice exhibit supernumerary teeth, both in the incisors and molars, and fused teeth in the molar teeth region. Because BMP-4 is known to be involved in the induction of the enamel knot signaling centers, loss of the inhibitory effect of USAG-1 might induce extra signaling centers, resulting in supernumerary teeth. Kassai *et al.*[17] independently reported that *USAG-1/ectodin* (they renamed USAG-1 as ectodin) knockout mice have enlarged enamel knots, altered cusp patterns, and extra teeth. They also reported that excess BMP accelerates patterning in *USAG-1*-deficient teeth, and proposed that USAG-1 is critical for robust spatial delineation of enamel knots and cusps.[17]

Another aspect of USAG-1 and sclerostin: link between BMP and Wnt pathway

Based on amino-acid sequence similarity, USAG-1 and sclerostin (see Discovery and characterization of USAG-1) seem to form a new family of BMP antagonists (Figure 1c).[11]

Sclerostin was first identified as BMP antagonist expressed in the bones, but so far there has been a controversy about its biological functions. Although Kusu *et al.*[18] and Winkler *et al.*[19] demonstrated that sclerostin binds BMP and inhibits alkaline phosphatase activity induced by BMP, van Bezooijen *et al.*[20] demonstrated that sclerostin cannot inhibit early BMP response, in spite that they approved that sclersotin binds BMPs and antagonizes their bone-forming capacity. On the other hand, Li *et al.*[21] showed that sclerostin binds Wnt co-receptor, lipoprotein receptor-related protein 5/6 (LRP5/6), and antagonizes canonical Wnt pathway, whereas Winkler *et al.* demonstrated that sclerostin inhibition on Wnt-induced cell differentiation is indirect and mediated by BMP.

Recently, Itasaki *et al.*[22] reported that wise/USAG-1 (they renamed USAG-1 as wise) functions as a context-dependent activator and inhibitor of Wnt signaling in *Xenopus* embryogenesis, as well as the physical interaction between wise/USAG-1 and LRP6.

Further studies are needed to clarify the biological function of USAG-1 and sclerostin; however, it might be possible that these two proteins possess dual activities, and play as a molecular link between Wnt and BMP signaling pathway.

KIELIN/CHORDIN-LIKE PROTEIN

Kielin/chordin-like protein (KCP) is a secretory protein with 18 cysteine-rich chordin repeats, and recently, Lin *et al.*[23] found that KCP increases the binding of BMP-7 to its receptor and enhances downstream signaling pathways. The expression of KCP was detected in developing nephrons and diseased kidney, but not in adult healthy kidneys. They demonstrated that $KCP^{-/-}$ mice are susceptible to tubular injury and interstitial fibrosis, and concluded that KCP attenuates renal fibrosis, and could be a target for therapeutic trials.

CONCLUSION

In conclusion, BMP-7 and its modulators play important roles in the progression of renal diseases. Because negative and positive modulators of BMP signaling regulate and define the boundaries of BMP activity, further understanding of these modulators would give valuable information about their pathophysiological functions and provide a rationale for a therapeutic approach against these proteins.

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