Modulator of bone morphogenetic protein activity in the progression of kidney diseases

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Tubular damage and interstitial fibrosis is a final common pathway leading to end-stage renal disease, and once tubular damage is established, it cannot be reversed by currently available treatment. The administration of bone morphogenetic protein-7 (BMP-7) in pharmacological doses repairs established tubular damages and improves renal function in several kidney disease models; however, pathophysiological role of endogenous BMP-7 and regulatory mechanism of its activities remain elusive. The activity of BMP is precisely regulated by certain classes of molecules termed BMP agonist/antagonist. In this review, roles of BMP agonist/antagonists possibly modulating the activity of BMP in kidney diseases are discussed. Our group demonstrated that uterine sensitization-associated gene-1 (USAG-1), a novel BMP antagonist abundantly expressed in the kidney, is the central negative regulator of BMP-7 in the kidney, and that mice lacking USAG-1 (*USAG-1*$^{-/-}$ mice) are resistant to kidney injuries. *USAG-1*$^{-/-}$ mice exhibited markedly prolonged survival and preserved renal function in acute and chronic renal injuries. 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 is attributed to enhancement of endogenous BMP-7 signaling. Furthermore, the administration of neutralizing antibody against BMP-7 abolished renoprotection in *USAG-1*$^{-/-}$ mice, indicating that USAG-1 plays a critical role in the modulation of renoprotective action of BMP, and that inhibition of USAG-1 will be promising means of development of novel treatment for kidney diseases.

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BMP-7 IN KIDNEY DISEASES

Bone morphogenetic proteins (BMPs) are phylogenetically conserved signaling molecules that belong to the transforming growth factor-β superfamily. Although these proteins were first identified by their capacity to promote endochondral bone formation, they are involved in the cascades of body patterning and morphogenesis. Furthermore, BMPs play important roles after birth in the pathophysiology of several diseases, including osteoporosis, arthritis, pulmonary hypertension, cerebrovascular diseases, and cancer and kidney diseases.

BMP-7, also known as osteogenic protein-1, is a 35-kDa homodimeric protein, and kidney is the major site of BMP-7 synthesis during embryogenesis as well as postnatal development.[1] Its genetic deletion in mice leads to severe impairment of kidney development, resulting in perinatal death.[2,3] Expression of BMP-7 in adult kidney is confined to distal collecting tubules and podocytes of glomeruli,[4] and the expression decreases in several kidney disease models, including acute ischemic renal injury, tubulointerstitial fibrosis, diabetic nephropathy, and remnant kidney model.[5] Recently, several reports indicate that the administration of pharmacological doses of BMP-7 inhibits and repairs acute and chronic renal injury in animal models.[6–8] The administration of BMP-7 reverses transforming growth factor-β1-induced fibrogenesis and epithelial-to-mesenchymal transition (EMT) and induces mesenchymal-to-epithelial transition *in vitro*,[8] inhibits the induction of inflammatory cytokine expression,[4] attenuates inflammatory cell infiltration, and reduces apoptosis of tubular epithelial cells in renal disease models. Collectively, BMP-7 plays critical roles in repairing processes of the renal tubular damage in kidney diseases. However, the physiological role and precise regulatory mechanism of endogenous BMP-7 activity remain elusive.

REGULATORY MECHANISM OF BMP ACTIVITY

The local activity of endogenous BMP is controlled by at least three different mechanisms. First, the expression pattern of BMP and its cell surface receptors controls local activity of BMP. Second, high-affinity binding of BMP to extracellular matrix increases its local concentration. Vukicevic *et al.*[9] previously showed that BMP-7 binds to basement membrane

components including type IV collagen. In addition, Gregory *et al.*[10] recently demonstrated that the prodomain of BMP-7 targets BMP-7 complex to the extracellular matrix. In most tissues, *bmp* mRNA expression and BMP protein are found colocalized.[9] Restricted diffusion of BMP proteins should increase its local concentration.

Finally, BMP signaling is precisely regulated by certain classes of molecules termed as BMP antagonists.[11] BMP antagonists function through direct association with BMPs, thus prohibiting BMPs from binding their cognate receptors. The interplay between BMP and their antagonists fine-tunes the level of available BMPs, and governs developmental and cellular processes as diverse as establishment of the embryonic dorsal–ventral axis, induction of neural tissue, formation of joints in the skeletal system, and neurogenesis in the adult brain. The indispensable roles of BMP-7 in the kidney led us to postulate the existence of some BMP antagonist that modulates the activities of BMP-7 in the kidney.

GREMLIN: BMP ANTAGONIST WITH A ROLE IN KIDNEY DEVELOPMENT

Gremlin was identified from a *Xenopus* ovarian library for activities inducing secondary axis, and it encodes 28-kDa protein that binds to BMP-2/4 and inhibits their binding to the receptors.

Gremlin knockout mice are neonatally lethal because of the lack of kidneys and septation defects in the lung. Gremlin is expressed in metanephric mesenchyme surrounding ureter tips, and gremlin-mediated BMP antagonism is essential to induce metanephric kidney development.[12]

Gremlin is also known as IHG-2 (induced in high glucose 2) because its expression in cultured kidney mesangial cells is induced by high ambient glucose, mechanical strain, and transforming growth factor-β.[13] The expression of gremlin in adult kidney is almost undetectable in healthy status, but the expression increases in several kidney disease models, including diabetic nephropathy,[13] cisplatin nephrotoxicity,[14] and unilateral ureteral obstruction. However, the role of gremlin in the progression of kidney diseases remained to be elucidated.

NOGGIN

Noggin is a 32 kDa glycoprotein secreted by Spemann organizer of *Xenopus* embryos, and is found to rescue dorsal development in the ultraviolet-induced ventralized embryos. Noggin binds to BMP-2 and BMP-4 with high affinity and to BMP-7 with low affinity, and prevent BMPs from binding to its receptors. In mice, noggin is expressed in the node, notochord, dorsal somite, condensing cartilage, and immature chondrocytes, and is essential in skeletal and joint development.

Recently, it is reported that overexpression of noggin in podocytes leads to the development of mesangial expansion, indicating the importance of endogenous BMP signaling in the maintenance of glomerular structure. Because the expression of noggin is almost undetectable in healthy and diseased kidney, other negative regulator of endogenous BMP might play a role in glomerular mesangial expansion.

USAG-1 AS A NEGATIVE REGULATOR OF BMP IN THE KIDNEY

Discovery and characterization of USAG-1

Through a genome-wide search for kidney-specific transcripts, our group found a novel gene, which encodes a secretory protein with a signal peptide and cysteine-rich domain.[15] The rat ortholog of the gene was previously reported as a gene of unknown function that was preferentially expressed in sensitized endometrium of rat uterus, termed uterine sensitization-associated gene-1 (USAG-1). Amino-acid sequences encoded in rat and mouse cDNAs are 97 and 98% identical to the human sequence, respectively, indicating high degrees of sequence conservation.

Domain search predicted this protein to be a member of the cystine-knot superfamily, which comprises of growth factors, BMPs, and BMP antagonists. Homology search revealed that USAG-1 has significant amino-acid identities (38%) to sclerostin, the product of the *SOST* gene. Mutations of *SOST* are found in patients with sclerosteosis, a syndrome of sclerosing skeletal dysplasia. Because sclerostin was subsequently shown to be a new member of BMP antagonist expressed in bones and cartilages, USAG-1 is postulated to be a BMP antagonist expressed in the kidney.

USAG-1 protein is a 28–30 kDa secretory protein and behaves as a monomer, in spite that a number of BMP antagonists form disulfide-bridged dimers.[15,16] This is consistent with the fact that USAG-1 protein does not have the extra cysteine residues present in noggin and differential screening-selected gene aberrative in neuroblastoma (DAN), which are necessary to make inter-molecular disulfide bridges. Recombinant USAG-1 protein physically interacts with BMP-2, -4, -6, and -7, leading to the inhibition of alkaline phosphatase activities induced by each BMP in C2C12 cells and MC3T3-E1 cells dose-dependently,[15,16] whereas sclerostin only inhibits BMP-6 and BMP-7 activities.

Furthermore, the activity of USAG-1 as a BMP modulator was confirmed *in vivo* using *Xenopus* embryogenesis. Injection of synthetic RNA encoding BMP antagonists to the ventral portion of *Xenopus* embryos inhibits the ventralizing signal of endogenous BMP, and induces dorsalizing phenotypes of the embryos, including secondary axis formation and hyperdorsalization. The injection of as little as 100 pg USAG-1 mRNA was sufficient to cause secondary axis formation, and injection of increasing doses of mRNA up to 1000 pg led to a corresponding increase in the frequency of dorsalization phenotypes, whereas embryos developed normally when irrelevant mRNA was injected.

Expression of USAG-1

In mouse embryogenesis, expression of USAG-1 mRNA was first detected on E11.5 and increased toward E17.5.[15] *In situ* hybridization of mouse embryos on E11.5 revealed moderate expression of USAG-1 mRNA in branchial arches and

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

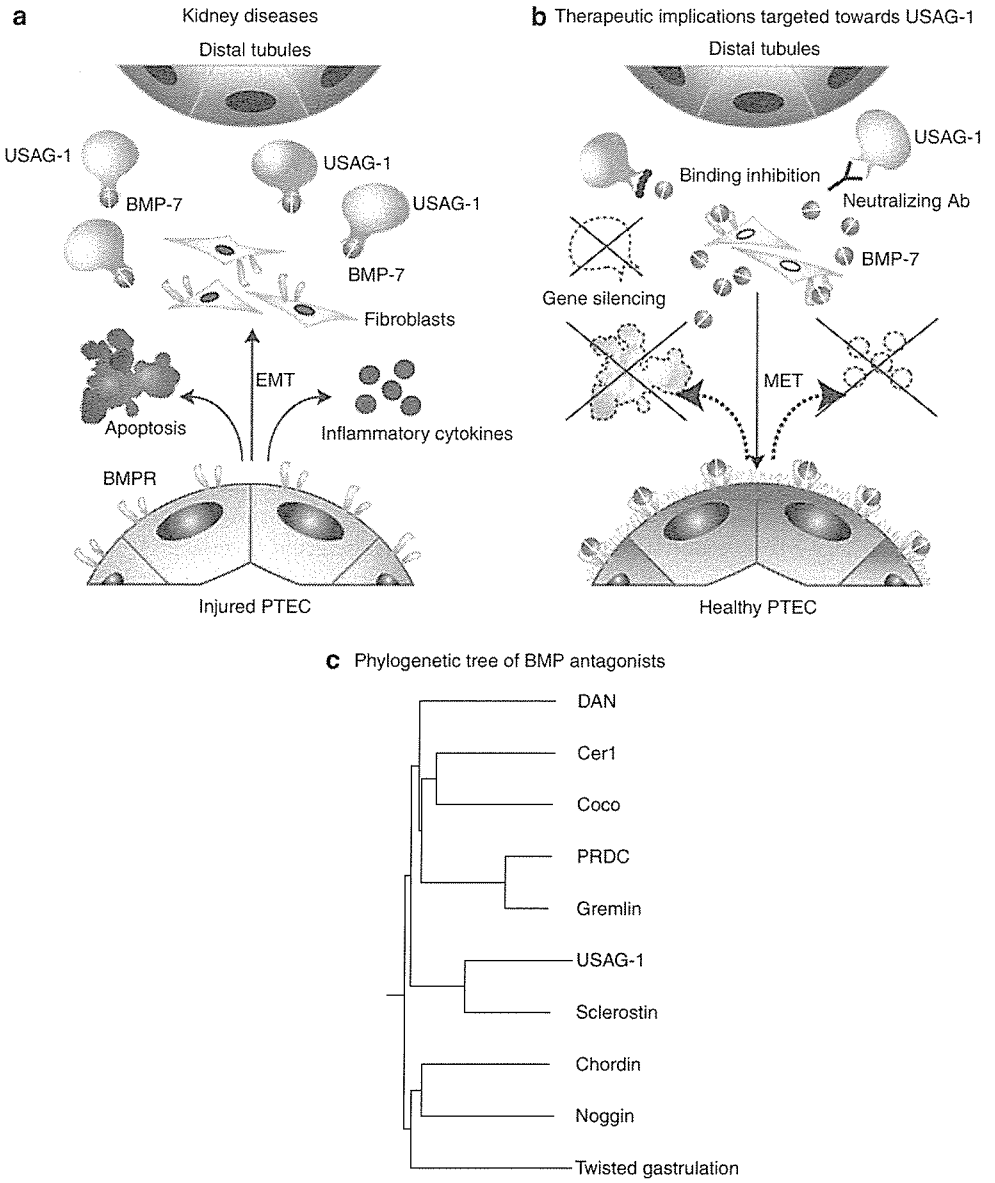

Figure 1 | Working hypothesis. (a) In kidney diseases, injured PTECs undertake apoptosis and EMT, and produce inflammatory cytokines. BMP-7 secreted from distal tubules is known to inhibit apoptosis, EMT, and production of cytokines of PTEC. USAG-1 is secreted from distal tubules, binds to BMP-7, and 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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2. BMP-7と腎臓特異的BMP拮抗分子USAG-1

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key words BMP, BMP antagonist, USAG-1, gremlin, twisted gastrulation, KCP, renal repair

動 向

腎不全の原因疾患の多くは糸球体疾患であるが，糸球体障害の程度よりも，むしろそれに続いて引き起こされる尿細管障害の程度が将来の腎機能低下を反映するといわれている．従来の治療法のほとんどは糸球体障害をターゲットにしたものであり，続発する尿細管障害を予防，あるいは，遅延させることはできるが，完成した尿細管障害を元に戻すことは難しい．また腎疾患は進行するまで無症状であるため，尿細管障害が完成された状況で発見されることのほうが多い．以上のことから，完成された尿細管障害を元に戻すことができるような薬剤があれば理想的であるが，現在のところそういった薬剤は存在しない．

近年薬理量のbone morphogenetic protein (BMP)-7を投与するといったん完成した腎障害が修復され，腎機能が回復することが報告された[1]．しかしながら内因性BMP-7の生理的機能およびその調節機構についてはいまだ明らかではない．本章ではBMPとその調節因子であるBMP antagonist/agonistに関する最近の知見を概説するとともに，筆者らが発見した新規腎臓特異的BMP拮抗分子USAG-1について紹介したい．

A. BMPとは

BMPは1965年骨形成因子として単離されたが，現在までに20種類以上のアイソフォームが報告され，いずれもTGFβスーパーファミリーに属することが知られている[2]．BMPの機能は，その発見の経緯とは裏腹に必ずしも骨に限局せず，発生段階の体軸決定や器官形成に重要な役割を果たすことが明らかとなってきた．BMPは大きな前駆体蛋白として翻訳され，その後二量体になる際にプロテアーゼによって切断され，mature formとなる．結晶解析の結果，単量体の中心にはTGFβスーパーファミリーに共通するcystine knotというモチーフがあり，6つのシステインがジスルフィド結合によって結び目のような構造を作っていることが明らかとなった[3]．分泌されたBMPは細胞膜表面に存在しtype I type IIのヘテロダイマーからなるセリンスレオニンキナーゼ型受容体であるBMP受容体を介して細胞内のSmadをリン酸化し，シグナルを伝達する．

B. BMP-7の腎疾患治療薬としての可能性

BMP-7（OP-1）は胎生期には広く発現しているが，ノックアウトマウスが腎形成不全のために

生後すぐに死亡することから腎発生に必須の因子であると考えられている[4,5]．BMP-7の発現は他臓器では生後低下するが，腎臓においては生後も強く発現し[6]，その発現は遠位尿細管および足細胞に限局する[7]．BMP-7の生理的機能はいまだ不明であるが，近年その腎障害修復における機能が明らかとなってきた．BMP-7の発現は虚血再灌流モデルや糖尿病性腎症，一側尿管結紮モデルなどで低下しており[8-11]，外来性に大量のBMP-7を投与することによって腎障害が予防されることが報告された[12-17]．さらに最近，抗基底膜抗体によって惹起される糸球体腎炎モデルにおいては，病変が形成されてからBMP-7を投与しても腎障害が修復され，腎機能が回復するということが報告された[1]．これは腎不全からの回復という点で画期的であり，ヒト腎不全治療への応用が待たれる．

BMP-7による腎保護機能の作用機序としては炎症性サイトカインの産生抑制や炎症性細胞浸潤抑制といった抗炎症作用[7]や，アポトーシスの抑制[18]などに加えて，TGF-β1による近位尿細管上皮細胞のepithelial-to-mesenchymal transition（EMT）を抑制し[1]，mesenchymal-to-epithelial transition（MET）を促進する[19,20]ことが知られている．このように内因性のBMP-7はTGF-β1と拮抗的に作用することによって腎臓の障害と修復のバランスを取っている可能性がある．腎障害においては，TGF-β1が発現増加しBMP-7が発現低下することによってTGF-β1系のシグナルが優位になっているが[21]，外来性に大量のBMP-7を投与することによってそのバランスを正常化し，腎修復を促進している可能性がある．

主として遠位尿細管で産生されるBMP-7がどのようにして近位尿細管に到達し，そのEMTを抑制するかについてはいまだ明らかではない．BMP-7は血中にも存在するため[17,22]，血中に分泌されたBMP-7が近位尿細管に到達する可能性と，間質を介して近傍の近位尿細管に到達する可能性がある．最近，BMP-7のプロドメインがfibrillinなどの細胞外マトリックスに結合することが報告されているが[23]，この機能によって内因性のBMP-7が局地的に高濃度を保って働く可能性が示唆された．

しかしながら，外来性にBMP-7を全身投与すると，投与されたBMP-7の取り込みは腎よりもむしろ肝で多く，効率的でない[24]．さらにBMP-7のエフェクター細胞は全身に分布しているため，副作用が懸念される．また反復投与すると高率に中和抗体が産生されるため，長期投与における効果には疑問がもたれる．その問題点を解決する可能性があるのがUSAG-1である．

C. 腎障害におけるBMP antagonist

1. 新規BMP拮抗分子USAG-1とその治療への応用

BMPの機能はそれ自身の発現の濃度勾配によって制御されるだけではなく，BMP antagonistとよばれる一群の因子によってON/OFF調節される[2]．BMP antagonistはBMPと直接に結合しBMP受容体への結合を阻害することによって，その活性を抑制する．腎臓においてはBMP-7の機能が非常に重要であるため，腎臓特異的なその調節因子があるのではないかと考えられていたが，まだみつかっていなかった．筆者らはゲノムワイドな臓器特異的遺伝子検索の過程で見出したUSAG-1（uterine sensitization-associated gene-1）が新規腎臓特異的BMP antagonistであることを見出した[25]．

USAG-1はラットの妊娠子宮の腺管上皮において受精卵の着床直前のみに発現増加する遺伝子としてすでに知られていたが[26]，その機能は不明であった．USAG-1はBMPや他のBMP

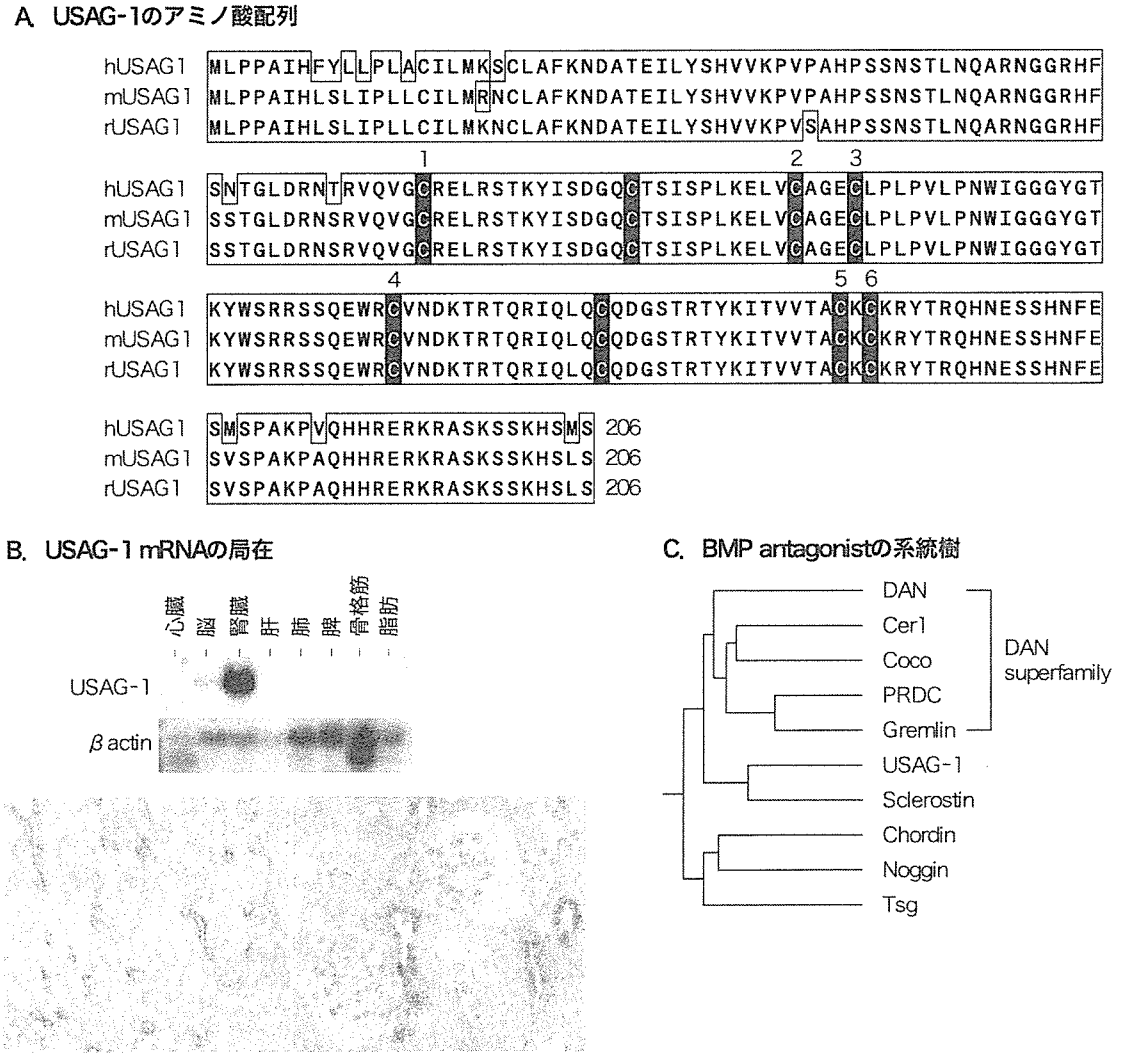

図1　WAG-1の構造，発現および相同性

antagonistと同様cystine knotスーパーファミリーの一員であり，そのアミノ酸配列はマウスとヒトで相同性98%ときわめてよく保存されている(図1A)[25]．さらにホモロジーサーチの結果，USAG-1は骨特異的BMP antagonistであるSclersotin[27]とアミノ酸配列で38%の相同性があり，類似の機能をもつのではないかと考えられた．柳田らはリコンビンナントUSAG-1を用いて，USAG-1がBMPと結合し，BMPによるアルカリフォスファターゼ誘導活性を阻害することを証明した[25]．さらにアフリカツメガエルの受精卵を用いた系では，USAG-1 RNAを腹側に打ち込むと内因性BMPによる胚の腹側化作用に拮抗して胚の背側化が起こることを証明した．以上のことからUSAG-1はin vitro，in vivoにおいてBMP antagonistとして働くことが示された．さらにそのligand特異性はBMP間であまり差がなかった．

多くのBMP antagonistが発生段階で強発現しても生後は発現低下していくのとは裏腹に，

USAG-1の発現は発生段階の後期に向けて急激に増加し，胎生17.5日ではその発現は腎臓の尿細管および歯のエナメル芽細胞に強く認められた．さらに成体の組織においては腎臓において最も強く発現しており，その発現は遠位尿細管に限局していた（図1B）．この局在はBMP-7と類似している[7]ことから，USAG-1は成体内においてもBMP-7の機能を調節しているのではないかと考えられる．さらに我々はUSAG-1ノックアウトマウス（以下USAG-1 KO）を作成し，USAG-1 KOが腎障害に対して抵抗性であることを証明した．この結果から，USAG-1の中和抗体やUSAG-1とBMP-7の結合阻害剤，あるいはUSAG-1の発現抑制剤などには腎疾患治療薬としての可能性があり，USAG-1の発現が腎臓特異的であることからBMP-7の投与に比べ副作用が少ないことが期待される．腎臓には他のBMP antagonistも発現しているが（後述），USAG-1はその中で最も発現が強い[28]．今後USAG-1の発現制御機構の解明が待たれる．

同じ頃Avsian-Kretchmerらはバイオインフォマティクスの手法を用いてcystine knotをもつ新規分泌蛋白を検索し，USAG-1がBMP antagonistである可能性を提示した[3]．彼らはsclerostinとUSAG-1のアミノ酸配列が他のBMP antagonistよりも近傍に位置することからこの二つが新しいBMP antagonistのファミリーを形成する可能性を示唆した（図1C）．USAG-1とsclerostinは，fuguでは単独のorthologueをもつのみであり，その後の進化の過程で2つの分子に分かれた可能性がある．さらに他のBMP antagonistが二量体で存在するのに対しUSAG-1とsclerostinは単量体で存在するといった共通点があり，これらの知見はこの2分子が新しいファミリーを形成するというAvsian-Kretchmerらの仮説を裏づけるものである．

さらにItasakiらはツメガエルのアニマルキャップアッセイを用いてUSAG-1（Wiseと命名）がWntのco-receptorであるLRP6に結合してWntシグナルを修飾することを証明した[29]．腎障害におけるWntの機能はBMPほどには明らかになっていないが，腎虚血再灌流モデルや葉酸投与モデルにおいてWnt4の発現が増加することが報告されており[30,31]，USAG-1/WiseがBMPシグナルとWntシグナルをつなぐ鍵因子である可能性を含め，今後の検討が期待される．

2. gremlin

gremlinはもともとアフリカツメガエルの卵巣のlibraryから二次軸形成を誘導する因子として同定されたが[32]，その後DANスーパーファミリーに属するBMP antagonistであることが明らかになった[32]（図1C）．gremlinノックアウトマウスは腎・肺形成不全のために生後すぐ死亡するが，その他にも四肢の形成異常が認められる[33,34]．gremlinは腎メサンギウム細胞を高グルコースやメカニカルストレッチ，TGF-β1で刺激した際に発現増加することが知られており，IHG-2(induced in high glucose 2) ともよばれている[35]．gremlinは正常の腎臓ではほとんど発現していないが(unpublished data) ストレプトゾトシンで誘導した糖尿病性腎症においては発現増加することが知られている[11,36]．

3. twisted gastrulation (Tsg)

TsgはBMP antagonistの中でも特殊であり，BMP antagonistであるchordinとBMPと三量体を作る．さらにその三量体を形成したchordinはメタロプロテアーゼであるtolloidによって切断される[37-39]．Tsgはこの三量体の形成を促進し，BMPと受容体の結合を阻害するという点においてはBMP antagonistである．しかしながらいったん三量体を形成すると，Tsgの存在によってchordinがtolloidによる切断を受けやすくなり，

切断されたchordinからBMPが離れると受容体に結合できるようになる．この点においてTsgはBMP agonistとして働くと考えられる．このようにTsgの機能は双方向性であり[40]，その制御機構の解明が待たれる．Tsgノックアウトマウスは正常に生まれるが，内軟骨骨化障害による成長遅延，Tリンパ球分化障害，糸球体の成熟障害が認められ，生後1カ月程度で死亡する[41]．Tsgの発現は生後の腎臓でもUSAG-1ほどではないものの比較的強く認められ[28]，その生理的機能については今後の検討が待たれる．

4. KCP

近年腎臓においてBMPの機能を促進する因子kielin/chordin-like protein（KCP）が発見された[42]．KCPはシステインリッチドメインをもつ分泌蛋白であり，BMPと結合してtype I受容体への結合を強め，その作用を促進する．KCPの発現は胎生期の中腎管や後腎の近位尿細管などに認められる．生後の腎臓ではほとんど発現していないが，一側尿管結紮モデルにおいては結紮側，対側ともにKCPの発現が増加する．KCPのノックアウトマウスは正常に生まれてくるが，一側尿管結紮モデル，葉酸投与モデルを惹起すると間質の線維化が促進されることが報告されている．以上のことからKCPが腎臓の線維化に対して抵抗性に働く可能性が示唆された．

むすび

腎障害修復におけるBMPの作用は，抑制的に働くUSAG-1，gremlin，促進的に働くKCP，双方向性に働くTsgなどによって多面的に調節されていると考えられる．その制御機構を明らかにすることが今後の腎疾患治療薬の開発の上で重要である．これまでBMP/BMP antagonistの生後の機能解析はあまり進んでいなかった．その原因としてはこれらの遺伝子のノックアウトマウスが胎生期致死や発生異常をきたし，生後の機能解析に不向きであったことが大きい．今後コンディショナルノックアウトマウスと臓器特異的Creマウスを用いた研究でこれらの遺伝子の機能解析が進むことが期待される．

腎臓の修復過程には発生，分化の過程によく似た経過がしばしば認められる[43]．発生段階で分化を促進したBMPとその調節因子が生後の腎障害における脱分化の過程で再びどのような機能を果たすのか，今後の検討を待ちたい．

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腎臓内科学

新規BMPアンタゴニストであるUSAG-1は腎不全治療薬のターゲットである

USAG-1 as a therapeutic target for kidney diseases

従来の腎疾患治療薬は予防的で，いったん腎不全に陥った腎を元どおりに戻す薬剤は存在しない．近年，薬理量の bone morphogenetic protein(BMP)-7 を投与するといったん完成した腎障害が修復され，腎機能が回復することが報告された[1]．しかし，BMP-7 の受容体は腎に限局せず，広範な副作用が懸念される．本稿では著者らが発見した新規腎特異的 BMP アンタゴニスト，USAG-1(uterine sensitization-associated gene-1)について紹介するとともに，その腎不全治療薬ターゲットとしての可能性について概説する．

BMP-7の腎疾患治療薬としての可能性

BMP-7 は，ノックアウトマウスが腎形成不全のために生後すぐに死亡することから，腎発生に必須の因子であると考えられている[2,3]．BMP-7 は生後の腎にも強く発現し，その発現は各種腎疾患モデルマウスで低下する．近年，これら腎疾患モデルに大量のリコンビナント BMP-7 を投与すると腎障害が予防されることが報告された．さらに，特定の糸球体腎炎モデルにおいては病変が形成されてから BMP-7 を投与しても腎障害が修復され，腎機能が回復するということが報告された[1]．これは腎不全からの回復という点で画期的であるが，BMP-7 のエフェクター細胞は全身に分布しているため，副作用が懸念される．また，反復投与すると高率に中和抗体が産生されるため，長期投与における効果には疑問がもたれる．その問題点を解決する可能性があるのが USAG-1 である．

新規BMPアンタゴニストUSAG-1の発見とその治療への応用

BMP の機能はそれ自身の発現の濃度勾配によって制御されるだけではなく，BMP アンタゴニストとよばれる一群の因子によって ON/OFF 調節される．BMP アンタゴニストは BMP と直接に結合し BMP 受容体への結合を阻害することによって，その活性を抑制する．腎においては BMP-7 が重要な役割を果たすため，腎特異的なその調節因子があるのではないかと考えられていた．

著者らはゲノムワイドな臓器特異的遺伝子検索の過程で，新規腎特異的 BMP アンタゴニストである USAG-1 を見出した[4]．USAG-1 は腎にほぼ特異的に発現し，その発現は遠位尿細管に限局する．この局在は BMP-7 と類似していることから，USAG-1 は成体内においても BMP-7 の機能を調節しているのではないかと考えられた．

著者らは USAG-1 遺伝子欠損マウスを用いた解析で，このマウスが野生型マウスと比べて腎障害に抵抗性であること，その腎障害抵抗性は内因性 BMP-7 の活性増強を介していること，USAG-1 が腎に発現する BMP アンタゴニストのなかでもっとも多いことを証明した[5]．

この結果から USAG-1 の中和抗体や USAG-1 と BMP-7 の結合阻害剤，あるいは USAG-1 の発現抑制剤などには腎疾患治療薬としての可能性があり，これらの薬剤は USAG-1 の発現が腎特異的であることから，BMP-7 の投与に比べ副作用が少ないことが期待される．

さらに Itasaki らは，USAG-1 (Wise と命名)が Wnt の co-receptor である LRP6 に結合して Wnt シグナルを修飾することを証明した[6]．腎障害における Wnt の機能は BMP ほどには明らかになっていないが，腎虚血再灌流モデルや葉酸投与モデルにおいて Wnt4 の発現が増加することが報告されており，USAG-1/Wise が BMP シグナルと Wnt シグナルをつなぐ鍵因子である可能性を含め，今後の検討が期待される．

おわりに

腎の修復過程には，発生，分化の過程によく似た経過がしばしば認められる．発生段階で分化を促進した BMP とその調節因子が，生後の腎障害における脱分化の過程でふたたびどのような機能を果たすのか検討していきたい．

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