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Bone morphogenetic proteins and growth differentiation factors as drug targets in cardiovascular and metabolic disease

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Bone morphogenetic proteins (BMPs) and growth differentiation factors (GDFs) control the development and homeostasis of multiple tissue types in many organisms, from humans to invertebrates. These morphogens are expressed in a tissue-specific manner and they signal by binding to serine–threonine kinase receptors, resulting in coordinated changes in gene expression that regulate the differentiation and development of multiple tissue types. In addition, these proteins are regulated post-transcriptionally through binding to several soluble proteins. In this review we focus on a subset of BMPs and GDFs that have been implicated in the pathophysiology of type 2 diabetes and cardiovascular disease.

Bone morphogenetic proteins (BMPs) and growth differentiation factors (GDFs) are members of the transforming growth factor-β (TGF-β) superfamily (Figure 1). This superfamily comprises a large number of structurally related proteins that regulate many cellular processes, including cellular proliferation, differentiation and lineage determination. These factors are expressed in a tissue-specific manner and control the development and homeostasis of tissues in organisms ranging from drosophila to humans. The ability to modulate growth, repair and differentiation of a variety of tissue types has generated significant interest in exploiting these pathways for therapeutic use and one of the earliest identified BMPs, BMP-2, has been approved for lumbar spinal fusions in humans. Because these family members play such an important developmental role in regulating tissue growth and differentiation, caution must be exercised to be certain that similar effects are also seen in adult animals. This is a crucial point to consider when assessing the potential therapeutic benefit gained through modulation of these developmental signaling pathways. In this review we will focus on the roles of various BMPs and GDFs in cardiovascular disease and type 2 diabetes.

Signaling by BMPs and GDFs

BMPs and GDFs signal by binding to receptors of the serine–threonine kinase class. There are two types of receptors, type I and type II, each containing an extracellular ligand-binding domain, a transmembrane domain and an intracellular serine–threonine kinase domain. Two modes of binding have been reported (Figure 2). First, classical members of the BMP family, best characterized by BMP-2 and BMP-4, where the ligand binds with high affinity to the type I receptor and with weak affinity to the type II receptor, resulting in the formation of a ternary complex [1]. The second mode of binding is best exemplified by the TGF-β–activin class, which includes GDF-8 (myostatin), where the ligand binds with high affinity to the type II receptor but does not bind to the isolated type I receptor [2,3]. In this case, binding is an ordered process involving a high-affinity interaction of the ligand to the type II receptor followed by recruitment of the type I receptor to form the ternary complex. Following formation of the ternary complex, the type II receptor kinase transphosphorylates multiple serine and threonine residues within the GS domain (a Gly–Ser-rich sequence located in the intracellular juxtamembrane region of the receptor kinase) of the type I receptor, resulting in activation of its serine–threonine kinase domain. In turn, the activated type I receptor can phosphorylate a family of R-Smad proteins (receptor-regulated Smads) that can homotrimerize and form complexes with the Co-Smad, Smad4. These complexes then translocate to the nucleus where they can activate transcription from several target genes. It is of note that there are only a small number of receptors, 12 in total, mediating the signaling of a large family of ligands, 42 in total, resulting in overlapping signaling between factors. Therefore, specificity of therapeutic agents will be best attained by targeting at the level of the ligand.

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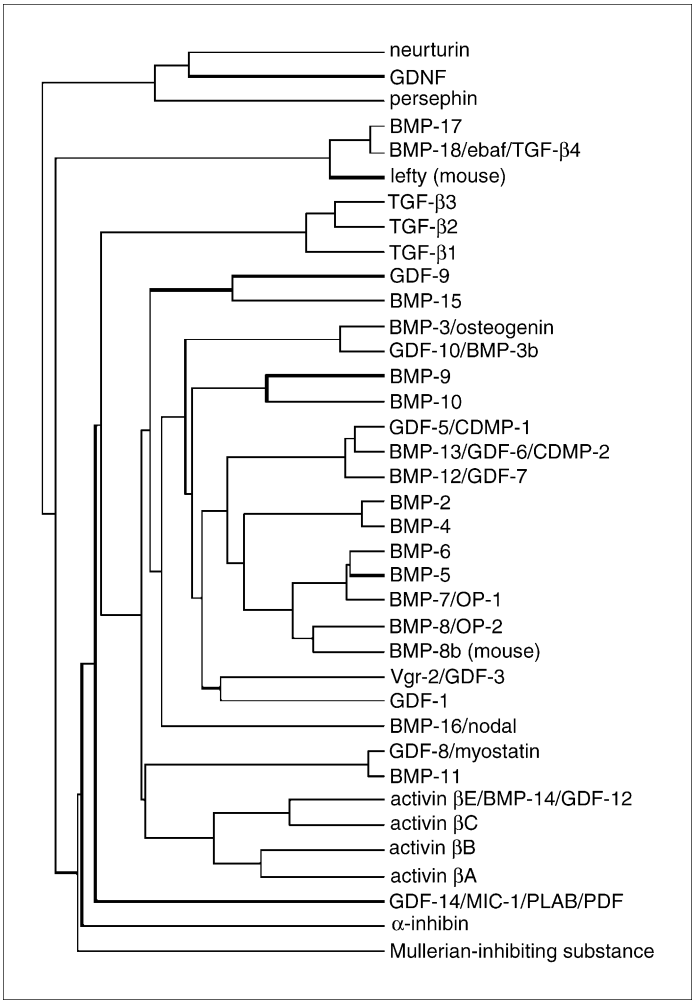

FIGURE 1

The transforming growth factor-β (TGF-β) superfamily. A dendrogram depicting the sequence relationships and diversity of this large gene-family of growth differentiation factors. The figure was generated using the sequences of the C-terminal cysteine-rich region of the TGF-β superfamily members, which were aligned using the PILEUP program (Genetics Computer Group, Madison, WI). Human peptide sequences were used, except where indicated in parenthesis.Abbreviations: BMP, bone morphogenetic protein; GDF, growth differentiation factor; GDNF, glial cell-line-derived neurotrophic factor; CDMP, cartilage-derived morphogenetic protein; OP, osteogenic protein; Vgr, Vg-1-related protein; MIC, macrophage inhibitory cytokine; PLAB, placental bone morphogenetic protein; PDF, prostate-derived factor.

BMPs and GDFs are also regulated at the receptor level by molecules that serve as ligand traps, preventing access of the ligands to their cognate signaling receptors. These antagonists include the soluble, high-affinity binding proteins follistatin, noggin, chordin, gremlin, DAN and growth and differentiation factor-associated serum protein-1 (GASP-1). In addition to the soluble factors that block access to the receptors there are decoy receptors, such as BMP and activin membrane-bound inhibitor (BAMBI), that lack a functional signaling domain and, thus, act in a dominant-negative capacity, as well as several proteins, such as betaglycan and cripto, that serve as co-receptors to facilitate an increase in binding affinity to the functional signaling receptors. In certain cases, including TGF-β and GDF-8, the propeptide region remains noncovalently associated with the mature ligand, holding it in a latent state. Understanding the mechanisms that regulate the activation of these latent complexes should also lead to new targets for therapeutic intervention.

Pulmonary hypertension

Primary pulmonary hypertension (PPH) is a rare but severe and progressive disease characterized by obstructive lesions of small pulmonary arteries and elevated pulmonary artery pressure, leading to heart failure and death. Disease development arises from vascular remodeling that involves structural changes in the wall architecture of the pulmonary arteries. Vessel-wall thickening, caused by the proliferation of smooth muscle and extracellular matrix deposition, leads to a decrease in lumen diameter and reduced capacity for vasodilatation. This results in increased pulmonary vascular resistance and, consequently, sustained pulmonary hypertension. Numerous mutations affecting BMP signaling have been associated in patients with PPH. Mutations in the BMP receptor type II (BMPR2) gene have been shown to underlie ~50% of all familial cases of PPH [4]. In sporadic cases of PPH, mutations in the BMPR2 gene have also been identified in up to 26% of the affected individuals. Patients developing pulmonary arterial hypertension (PAH) after exposure to the appetite suppressants fenfluramine and dexfenfluramine also have mutations in the BMPR2 gene [5,6]. A mechanistic link between familial and acquired pulmonary hypertension has been demonstrated in pulmonary arteriolar endothelial cells by the finding that angiopoietin-1 shuts off the expression of BMPR1A [also known as activin receptor-like kinase-3 (ALK-3)], a transmembrane protein required for BMP-2 signaling [7]. Du *et al.* [7] suggest that all forms of pulmonary hypertension are linked by defects in the signaling pathway involving angiopoietin-1, TIE2, BMPR1A and BMPR2. The BMPR2 cytoplasmic tail interacts directly with LIM kinase-1 (LIMK-1) and inhibits its ability to phosphorylate and inactivate cofilin, an actin depolymerizing factor. Mutations resulting in truncation of this cytoplasmic domain of BMPR2 are present in patients with PPH, suggesting that the deregulation of actin dynamics could contribute to the etiology of PPH [8]. The genes associated with many cases of PPH have been identified. Defects in the BMPR2 gene, as well as other genes in the signaling pathway such as ALK1, are partly responsible for PPH. However, the development of therapies based on this knowledge will depend upon further insights into the signaling pathways that are downstream of BMPR2.

Cardiovascular calcification

Cardiovascular calcification is a prominent feature of atherosclerosis and a common consequence of aging, diabetes, hypercholesterolemia and chronic renal insufficiency. Vascular smooth muscle cells (VSMCs) and vascular myofibroblasts contribute to the process of cardiovascular mineralization. Current data suggest that paracrine signals are provided by the osteoinductive factor, BMP-2, and might be responsible for the formation of vascular calcifications. In the aorta of individuals suffering from diabetes, there is an activated osteogenic program that includes expression of BMP-2 and the osteoblast homeobox-containing transcription factor, Msx2. Msx2 regulates osteogenic versus adipogenic differentiation of aortic myofibroblasts. Myofibroblasts capable of osteogenic and adipogenic differentiation can be diverted to the osteogenic lineage by BMP-2–Msx2 signaling and, thus, contribute to vascular calcification [9].

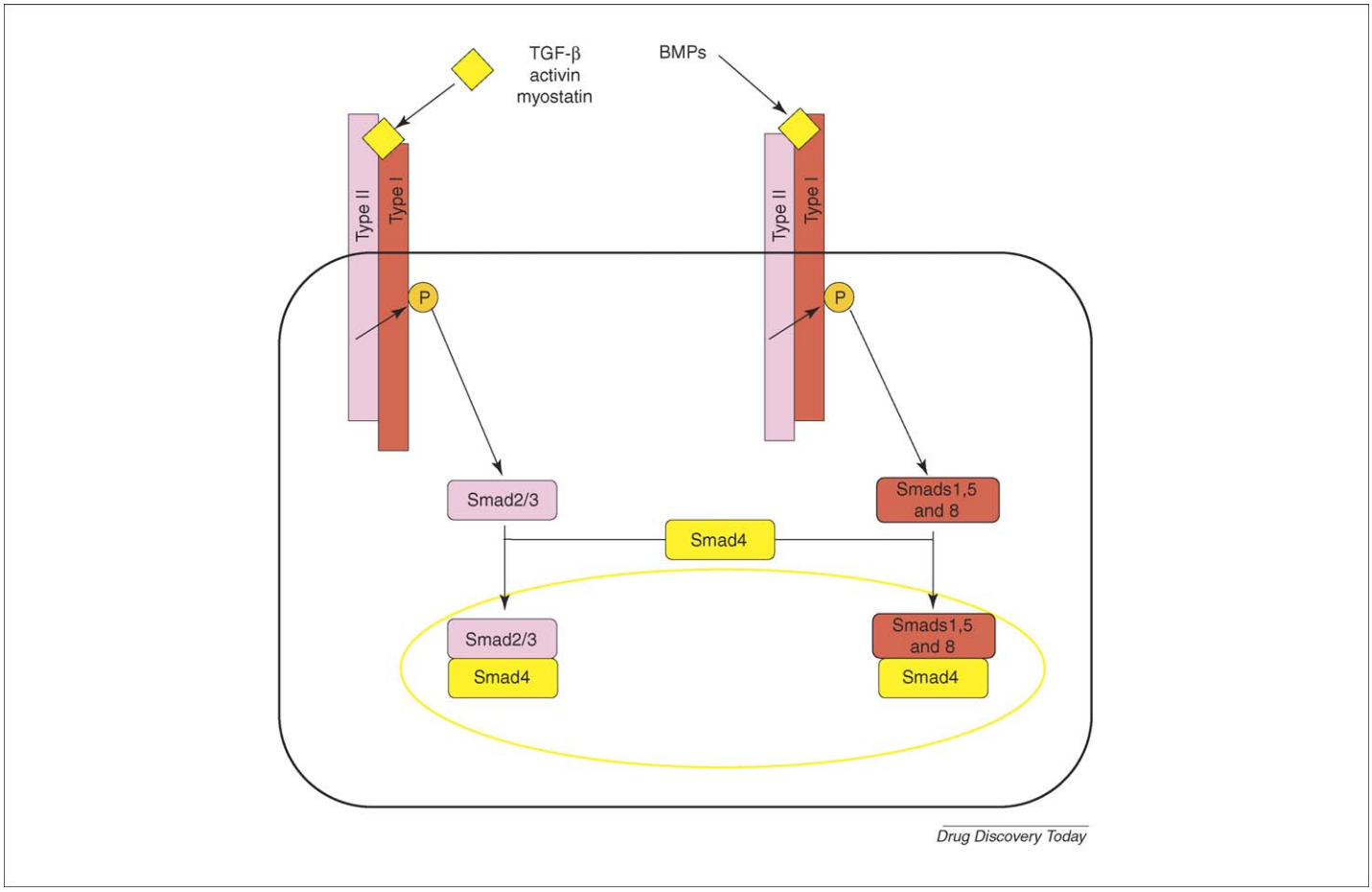

FIGURE 2

The two main signaling pathways of the transforming growth factor-β (TGF-β) superfamily. The ligands of this family of growth differentiation factors signal through two distinct, Smad-dependant, mechanisms. The TGF-β-like ligands (including activin and myostatin) bind in an ordered manner in which the ligand first binds with high affinity to the type II receptor, then recruits the low affinity type I receptor (ALK) to form a functional signaling complex. The BMP-like ligands bind directly to the type I receptor, but require activation by a type II receptor kinase to transduce a Smad-mediated signaling cascade. The TGF-β-like ligands signal through a Smad2/3 dependent mechanism, whereas the BMP-like ligands signal through Smads1, 5 and 8. Once activated through phosphorylation by the type I receptor kinase, the pathway-specific R-Smads form complexes with the Co-Smad4 and translocate to the nucleus to activate specific patterns of gene expression. Abbreviation: BMP, bone morphogenetic protein.

The vitamin K-dependent protein, matrix gamma-carboxylated glutamate protein (MGP), is a binding protein for BMPs. MGP-deficient mice develop extensive vascular calcification with replacement of the tunica media by progressively calcifying cartilage. A potential mechanism that explains these findings is inhibition of BMP-signaling by MGP. MGP has been shown to bind to BMP-2 and inhibit signaling [10]. Moreover, a form of MGP that was poorly gamma-carboxylated (and thus did not bind BMP-2) was identified by immunohistochemistry in calcified lesions in the aortic wall of aging rats. Therefore, arterial calcification could be a consequence of altered BMP-2 regulation by MGP.

The expression of regulators of bone formation and osteoclastogenesis in human atherosclerosis has also been examined [11]. Constitutive immunoreactivity of MGP, osteocalcin and bone sialoprotein (along with the absence of BMP-2, BMP-4, osteopontin and osteonectin) in healthy aortas and early atherosclerotic lesions has been observed. As atherosclerotic plaques develop calcification or bone formation, BMP-2, BMP-4, osteopontin and osteonectin are upregulated. BMP-2, -4 and -6 stimulate angiogenesis through osteoblast-derived vascular endothelial growth factor A [12–14]. BMP-7 [or osteogenic protein-1 (OP-1)] was also shown to induce angiogenesis [15]. BMP-4 protein expression was upregulated in endothelial cells exposed to oscillating shear stress, a proatherosclerotic environment. By contrast, laminar shear stress decreased BMP-4 expression [16]. In addition, BMP-4 expression was only found overlying foam cell lesions. Oscillating shear stress and BMP-4 induced intercellular adhesion molecule-1 (ICAM-1) expression and monocyte adhesion as well. Sorescu *et al.* [16] concluded that BMP-4 is a mechanosensitive inflammatory factor playing a crucial role in early steps of atherogenesis in the lesion-prone areas. BMP-7 deficiency has been shown to play a role in the progression of chronic renal failure, a condition that is often accompanied by vascular calcification, and treatment with BMP-7 is efficacious in several rodent models of renal disease (discussed later). BMP-7 treatment has been shown to prevent vascular calcification and reverse the upregulation of osteocalcin expression in a low-density lipoprotein (LDL) receptor null-mouse-model in which surgically induced uremia and high-cholesterol- and/or high-fat-diet-induced atherosclerosis led to chronic renal failure and vascular calcification [17].

Congestive heart failure

Several BMPs have been implicated in cardiac development and expression of these could play a role in congenital heart defects. BMP-10 appears to play a key role in cardiogenesis [18]. Studies in knockout mice have shown that BMP-10 increases the proliferative activity of cardiomyocytes at day E9.0–E9.5 of embryogenesis, and is also required to maintain normal expression of several key cardiogenic factors [including Nk-like homeobox gene 2.5 (Nkx2.5) and myocyte-enhancer factor 2C (MEF2C)] in the developing myocardium at mid-gestation [18]. The hearts of mice with a ventricular-restricted knockout of Nkx2.5 displayed no structural defects, but exhibited progressive heart block and massive trabecular muscle overgrowth [19]. A similar finding has been observed in humans carrying mutations in Nkx2.5 [20]. Transcriptional profiling studies show that BMP-10 is ectopically expressed in the adult ventricular myocardium and it has been suggested that BMP-10 is a major component of ventricular muscle defect [21].

BMP-4 has also been identified as a signal from the myocardium that directly mediates atrioventricular septation during development [22]. Defects in this process cause one of the most common human congenital heart abnormalities, atrioventricular canal defect (AVCD). Importantly, BMP-4 plays a role in apoptosis of capillary endothelial cells during development [23].

TGF-β and BMP-2 can upregulate the cardiac transcription factors Nkx2.5 and MEF2C in undifferentiated murine embryonic stem cells [24]. Embryoid bodies derived from these stem cells demonstrated an increased potential for cardiac differentiation and the authors of this review [24] conclude that embryonic stem cells are directed to differentiate into cardiomyocytes by signaling that is TGF-β–BMP-2 mediated.

The use of BMP antagonists has also demonstrated the importance of the BMP-signaling pathway in cardiogenesis. Using noggin to inhibit BMP activity, it has been demonstrated that BMP expression is required for Sox6 expression in cardiac differentiation, which might play a crucial role in the regulation of a cardiac L-type Ca^{2+} channel [25]. A novel BMP-binding protein [BMP-binding endothelial cell precursor-derived regulator (BMPER)], expressed by endothelial cell precursors, has BMP-antagonizing activity and might play a role in endothelial cell differentiation by modulating local BMP activity [26].

Results from immunohistochemistry on isolated heart sections demonstrated that myostatin (GDF-8) is localized in Purkinje fibers and cardiomyocytes [27]. Furthermore, following myocardial infarction, myostatin expression is upregulated in the cardiomyocytes surrounding the infarct area. Given that myostatin is expressed in fetal and adult hearts and that myostatin expression is upregulated in cardiomyocytes after the infarction, these data suggest that myostatin could play an important role in cardiac development and physiology.

GDF-15, known by many other names including macrophage inhibitory cytokine-1 (MIC-1) [28], placental transforming growth factor beta (PTGFβ) [29], prostrate-derived factor (PDF) [30] and placental bone morphogenetic protein (PLAB) [31], has recently been implicated in playing an important role in the progression of congestive heart failure [32,33]. Serum levels of GDF-15 are an independent risk indicator for adverse cardiovascular events [34]. Recent studies have demonstrated that in situations of ischemic injury, both in cultured cardiomyocytes and *in vivo* in models of coronary artery ligation, as well as in patients with acute myocardial infarction, GDF-15 mRNA and protein levels are highly upregulated. To elucidate the function of GDF-15 in heart disease, *gdf-15*-deficient mice were subjected to ischemia–reperfusion injury by transient coronary artery ligation [32]. Analysis of these mice indicated greater infarct size and increased cardiomyocyte apoptosis than that observed in wild-type littermates. Furthermore, treatment of cultured cardiomyocytes with recombinant human GDF-15 afforded protection from apoptosis and increased survival in response to ischemia–reperfusion [32]. Taken together, these results indicate that GDF-15 is cardioprotective, acting as a defense mechanism that limits myocardial tissue damage *in vivo*. GDF-15 protein administration or potentiation of cardiac-specific GDF-15-signaling pathways could lead to useful therapies in the prevention of congestive heart failure in patients with acute myocardial infarction.

Stroke

Stroke is one of the major causes of death and disability in the industrialized world and is manifested as ischemia or hemorrhage of one or more blood vessels of the brain. Herein, we focus on the roles of BMPs in ischemic stroke. BMP-7, given before middle cerebral artery occlusion (MCAO), reduces ischemic injury in the brain and improves the behavioral outcome [35–37]. Recent studies have indicated that receptors for BMP-7 were upregulated after brain ischemia [38], and it is possible that this upregulation could facilitate endogenous neurorepair in the ischemic brain.

Receptors for BMP-6 are found in the brain and are upregulated following injury [39]. Mild ischemia induced substantial changes in the expression of TGF-β1 and BMP-6 [40]. Pretreatment with BMP-6 before brain injury led to decreased ischemia-induced caspase-3 immunoreactivity, caspase-3 enzymatic activity and the number of TUNEL-positive cells in the ischemic cortex in BMP-6-treated animals [39]. These data suggest that BMP-6 can reduce ischemia–reperfusion injury by attenuating the molecular events underlying apoptosis.

BMP-7 in kidney development and homeostasis: implications in renal disease

Although several BMPs are expressed in overlapping patterns throughout kidney development, only BMP-7 has been demonstrated to play a crucial role in the proper formation of the kidneys. BMP-7 is expressed in the ureteric bud and the nephrogenic mesenchyme at 11.5 dpc in the mouse [41]. Targeted deletion of the mouse *BMP-7* gene results in postnatal lethality within 24 h of birth [42]. The mutant animals display bilateral dysplasia and hydroureter, ultimately dying as a result of renal failure. BMP-7 expression is highest in the kidney not only during development, but also in the mature animal, suggesting a continued role in tissue homeostasis. As in many adult tissue-repair situations, renal injury induces a recapitulation of the developmental progression of kidney morphogenesis. This paradigm has been a model for the therapeutic application of BMPs known to be involved in the development of specific organs. Experiments with recombinant human BMP-7 have demonstrated both antiapoptotic and anti-inflammatory effects in the adult kidney, allowing protection from various renal injuries, models of renal failure and complications resulting from chronic kidney diseases [43,44].

Models of acute renal failure exemplify the regenerative capacity of the liver through mechanisms similar to that observed during organogenesis. Systemic administration of BMP-7 has been demonstrated to be an effective means of regenerating kidney function in response to ischemia–reperfusion injury [44]. In the rat model, bilateral renal artery occlusion results in acute tubular necrosis and reduction in glomerular filtration rate, loss of renal function during the subsequent reperfusion, and inflammatory and cytotoxic injury also occurs. BMP-7 administered intravenously at 250 $\mu g\,kg^{-1}$, either before or after ischemia, followed by daily administration for three days following reperfusion, preserved kidney function. The protection from injury provided by exogenously administered BMP-7 occurs through several mechanisms, including inhibition of cell necrosis during ischemia along with suppression of inflammation and apoptosis during reperfusion. In another rat model of acute renal failure, in which injury is induced by nephrotoxins such as mercuric chloride and cisplatinum, BMP-7 protected kidney function and extended survival rates [45]. These studies indicate the potential of BMP-7 administration as a treatment for the tissue damage and loss of function induced in acute renal failure.

A sustained reduction in the glomerular filtration rate results in chronic renal failure, progression to end-stage renal disease and subsequent death in the absence of renal-replacement therapy, such as dialysis or kidney transplantation. Epithelial to mesenchymal transition, leading to tubulointerstitial fibrosis, are hallmarks in the progression of chronic renal disease. BMP-7 administration has shown efficacy toward these parameters in animal models of chronic renal failure. Unilateral ureteral obstruction (UUO) is a rodent model of renal injury that is characterized by progressive tubulointerstitial fibrosis and subsequent loss of renal function. Intraperitoneal administration of BMP-7 to rats (at does of 100 or 300 $\mu g\,kg^{-1}$) at the time of UUO and every other day thereafter diminished tubulointerstitial inflammation and fibrosis, resulting in the preservation of renal function [43]. In this study, the effects of BMP-7 administration were examined in comparison to that of the angiotensin-converting enzyme (ACE) inhibitor enalapril (ingested at 25 $mg\,kg^{-1}$ per day) in five day and ten day (sustained) UUO. The effect of BMP-7 in preventing tubulointerstitial fibrosis and preserving renal function was not only greater than that observed by enalapril treatment, but was achieved through a distinct mechanism – the prevention of tubular atrophy. Enalapril also results in a degree of renal protection in this model, indicated by the stimulation of renal blood flow, however this effect is mediated through the inhibition of angiotensin II activation of TGF-β signaling. Other studies demonstrate that BMP-7 can oppose TGF-β-induced epithelial to mesenchymal transition (EMT) in a Smad-dependent manner [46]. After demonstrating the counteractive effects of BMP-7 treatment on TGF-β-induced EMT expression in renal tubular epithelial cells and mammary ductal epithelial cells, as evidenced through restoration of E-cadherin expression, these authors further investigated this Smad-dependant reversal of TGF-β-induced EMT *in vivo*. A murine model of progressive chronic renal injury leading to tubulointerstitial disease associated with EMT and subsequent renal fibrosis, nephrotoxic serum nephritis (NTN), was examined. Recombinant human BMP-7 was administered to NTN mice by intraperitoneal injection at a concentration of 300 $\mu g\,kg^{-1}$ every other day during weeks 1–4 after nephrotoxic serum injection. BMP-7-treated mice were sacrificed at six weeks after nephrotoxic serum injection and compared with untreated mice that were sacrificed at different time points during weeks 1–6, to correlate the efficacy of BMP-7 treatment to the progression of the nephrotoxic-serum-induced renal disease. BMP-7 treatment initiated after one week of NTN prevented progression to chronic renal disease, whereas BMP-7 treatment initiated at either week 3 or week 4 of NTN almost completely reversed renal pathology. This reversal was characterized by a substantial decline in mortality and significant improvement in renal function at week 6, as indicated by blood urea–nitrogen and serum creatinine. As in the *in vitro* studies, EMT-associated progression of renal disease in the NTN-disease model was associated with a decrease in E-cadherin expression, which was restored by BMP-7 treatment. Examination of Smad2/3 (TGF-β-induced) and Smad1 (BMP-7-induced) localization in the *in vivo* studies support the BMP-7-induced reversal of TGF-β action through direct antagonism of pathway-specific Smad signaling. Additionally, BMP-7 treatment has been demonstrated to inhibit tubulointerstitial fibrosis and chronic renal disease in two genetic mouse models that mimic chronic renal disease, MRL/MpJ$^{lpr/lpr}$ lupus mice and α3-type IV collagen-deficient Col4A3$^{-/-}$ mice [47]. Recombinant BMP-7 treatment of the MRL/MpJ$^{lpr/lpr}$ mice inhibits tubulointerstitial fibrosis and progression of renal disease in a dose-dependent manner, whereas similar treatment of the Col4A3$^{-/-}$ mice prevents renal fibrosis and increases survival. Finally, BMP-7 has been implicated in playing an important role in the progression of diabetic nephropathy, a renal disease that is driven by hyperglycemia and culminates with end-stage renal failure. BMP-7 expression is downregulated in renal tubules during the evolution of diabetic nephropathy, consistent with its role in the preservation of tubular integrity. Administration of BMP-7 to streptazotocin-induced diabetic rats partially restores renal function, as evidenced by a reduction in renal hypertrophy and proteinuria, accompanied by restoring the glomerular filtration rate to nomal levels [48].

The involvement of BMP-7 in the preservation of renal function and resistance to injury is further supported by reports of BMP-7 antagonist regulation in the kidney. Gremlin, an extracellular protein which binds BMP-7 with high affinity and prevents signaling by blocking BMP-7 from binding to cellular receptors, has been shown to be upregulated in the diabetic glomerulus [49,50], whereas another recently identified BMP antagonist, uterine sensitization-associated gene-1 (USAG-1), has been shown to be highly expressed in the kidney [51]. Furthermore, USAG-1-deficient mice are resistant to renal injury in acute- and chronic-injury models, whereas administration of anti-BMP-7 neutralizing antibodies to USAG$^{-/-}$ mice abolished this renoprotection [52]. Taken altogether, the damaging fibrotic effects of TGF-β expression in the kidney, their opposition by BMP-7 and potentiation by BMP antagonists suggest multiple targets and distinct mechanistic approaches to design BMP–TGF-β-signaling modifying therapeutics, which hold great promise in the treatment of chronic kidney diseases.

Type 2 diabetes

The incidence of type 2 diabetes and the associated nutritional disorder, obesity, has increased greatly in the past century, primarily caused by changes in human behavior and lifestyle. Several

BMPs and GDFs have been implicated in metabolic diseases, and manipulation of these pathways might have therapeutic applications for obesity and type 2 diabetes. Mice that are deficient in myostatin, develop increased muscle mass and do not accumulate fat as they age [53]. Moreover, crossing the myostatin null allele into two different strains of obese mice resulted in mice with improved insulin sensitivity and reduced fat accumulation [54]. The exact mechanisms by which myostatin exerts its effect on glucose homeostasis are unknown. Administration of antimyostatin antibodies to mouse models of obesity will be useful in determining if the effects on glycemic control are developmental or whether they can be approached therapeutically.

BMP-9 was identified as a candidate molecule regulating glucose homeostasis in a functional genomic screen, using a secreted cDNA library and a battery of high-throughput cell-based assays [55]. BMP-9 is specifically expressed in the liver and receptors for BMP-9 have been identified on liver cells [56,57]. Administration of BMP-9 to hepatocyte cell lines reduced the expression of phosphoenolpyruvate carboxykinase (PEPCK), the rate-limiting enzyme in gluconeogensis, and administration of BMP-9 to myotubes activated Akt, a kinase involved in insulin signaling. In addition, injection of BMP-9 was shown to improve glycemic control in normal and diabetic mice, whereas intravenous administration of BMP-9 resulted in improved glucose tolerance and enhanced insulin sensitivity in Wistar rats [55].

Several groups have shown that myostatin, BMP-2, BMP-4 and BMP-7 have roles in regulating the differentiation of mesenchymal progenitors toward various lineages, including the adipogenic lineage [58–67]. In addition to these *in vitro* effects, GDF-3, originally identified and published as Vgr-2 [68,69], has recently been reported to be an adipogenic cytokine [70]. Overexpression of GDF-3 by adenoviral gene transfer results in profound weight gain on a high-fat diet but not on normal chow, suggesting that GDF-3 is active only under high-fat conditions. The mechanism of the high-fat diet-induced weight gain appears to be a direct effect on adipogenesis, with no effect on blood-glucose homeostasis or insulin resistance. The mice exhibit increased adipose tissue mass, adipocyte hypertrophy, hepatic steotosis and elevated leptin levels in plasma. Another report indicates that GDF-3 is upregulated in adipose tissue of FABP4/aP2 null mice on a high-fat diet [66]. Taken together these data suggest that GDF-3 could be an interesting target for metabolic disease indications, including obesity.

Conclusion

It is clear that BMPs and GDFs play important roles in the pathophysiology of several cardiovascular and metabolic diseases. Even though much knowledge has been gained, understanding the exquisite complexity of BMP and GDF biology remains a challenge. In terms of therapeutic approaches, inhibition of signaling using monoclonal antibodies or soluble receptors is feasible for those targets that have been validated *in vitro* [71,72]. However, manipulation of the signaling pathways represents a more complex undertaking given the overlapping signaling of this family of receptors.

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