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Review Article

Extracellular regulation of BMP signaling: welcome to the matrix

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Given its importance in development and homeostasis, bone morphogenetic protein (BMP) signaling is tightly regulated at the extra- and intracellular level. The extracellular matrix (ECM) was initially thought to act as a passive mechanical barrier that sequesters BMPs. However, a new understanding about how the ECM plays an instructive role in regulating BMP signaling is emerging. In this mini-review, we discuss various ways in which the biochemical and physical properties of the ECM regulate BMP signaling.

Introduction

Bone morphogenetic proteins (BMPs) belong to the superfamily of transforming growth factor-β proteins. More than 20 family members have been described. Initially identified for their osteogenic activity, BMPs play important roles in development and tissue homeostasis. Misbalances in BMP signaling are commonly observed in diseases such as cancer and fibrosis [1]. Therefore, tight extracellular and intracellular regulation of BMP signaling is critical.

BMPs are evolutionarily conserved secreted growth factors that, in vertebrates, are divided into several subgroups based on phylogenetic analysis [2] (Table 1). BMPs are translated as pre-proteins with a prodomain. Within the endoplasmatic reticulum, prodomains facilitate homo- and heterodimerization of BMPs via disulfide assembly [3]. Following secretion into the extracellular space, BMP dimers bind to BMP type II receptors, which then heterodimerize with type I receptors (Figure 1A,B). There are three type I receptors and three type II receptors (Table 1) [4]. Following heterodimerization, type I receptors are phosphorylated by type II receptors and subsequently activate the receptor-regulated R-Smads (Smad1, 5 and 8) through phosphorylation [5]. Phosphorylated Smad1, 5 and 8 heterodimers form a complex with the only common mediator Smad (Co-Smad) Smad4. Following nuclear translocation of the R-Smad/Co-Smad complex, BMP target gene expression is induced [5].

Intracellularly, BMP signaling is negatively regulated by the inhibitory Smads (I-Smads) Smad6 and Smad7, which compete with Smad4 for binding to R-Smads [6], or by Smad ubiquitin ligases of the Smurf family [7]. Extracellularly, a variety of secreted antagonists, such as noggin and chordin, bind to BMPs and inhibit their ability to interact with BMP receptors (BMPRs) (Figure 1C) [8]. BMP antagonists are classified into three families [9] (Table 1). These antagonists bind distinct BMPs with varying degrees of affinity and thereby selectively modulate BMP signaling responses [10]. Other extracellular BMP-binding proteins form soluble shuttling complexes with BMPs, fostering increased diffusion rates and ranges (Figure 1D,E and Table 1) [11]. The activity, bioavailability and diffusion of extracellular BMP signaling agonists and antagonists are further regulated by extracellular matrix (ECM) components [12].

The ECM is the noncellular compartment that surrounds cells, and includes basement membranes and the interstitial matrix. It comprises a variety of proteins and polysaccharides. Each tissue has its unique composition of ECM molecules. These components can often directly bind to and interact with growth factors, receptors and co-receptors through their biochemical properties [13]. On the one hand, these interactions can lead to simple sequestration of growth factors in the extracellular milieu.

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Table 1 Mammalian bone morphogenetic protein signaling molecules and their homologs in *Drosophila melanogaster*

The table is based on the NCBI HomoloGene database. Abbreviations: ACVR, activin A receptor; ALK, activin receptor-like kinase; BMP, bone morphogenetic protein; DAN, differential screening selected gene abberative in neuroblastoma; GDF, growth differentiation factor.

Mammalian	*Drosophila melanogaster*
BMPs	
BMP2/4	Decapentaplegic (Dpp)
BMP5/6/7/8	Glass bottom boat (Gbb), Screw (Scw)
BMP9/BMP10	
BMP12/13/14 (GDF5/6/7)	
BMPRs	
Type I receptors	
ACVR1 (ALK-2)	Saxophone (Sax)
BMPR1A (ALK-3)	Wishful thinking (Wit)
BMPR1B (ALK-6)	Thick veins (Tkv)
Type II receptors	
ACVR2A	Punt (Put)
ACVR2B	
BMPR2	
BMP antagonists	
Chordin (CHRD)	Short gastrulation (Sog)
Noggin (NOG)	
DAN family	
Gremlin 1 (GREM1)	
Cerberus 1 (CER1)	
Neuroblastoma 1 (NBL1)	
Protein related to DAN and cerberus (PRDC)	
BMP-binding proteins	
BMP-binding endothelial regulator (BMPER)	Crossveinless-2 (Cv-2)
Twisted gastrulation BMP signaling modulator (Twsg1)	Twisted gastrulation (Tsg)

On the other hand, ECM components can play an instructive role in signaling by controlling the level, location and kinetics of growth factor presentation to their cognate receptors [14]. Additionally, the ECM is a structural determinant with defined physical properties. The molecular composition of the ECM determines its stiffness, elasticity and porosity, which directly has an impact on the diffusion of macromolecules [15]. Cells are capable of sensing these physical properties and reacting with distinct cellular responses, for example modified differentiation, which is referred to as mechanosignaling [16].

In the following, we discuss the biochemical and physical properties of the ECM and their role in regulating BMP signaling. Figures 1 and 2 provide a direct comparison of the current view of BMP signaling without and with the ECM present, respectively.

Biochemical properties of the ECM: from passive sequestration to an instructive role in BMP signaling

Several ECM components are capable of binding directly to BMP signaling molecules and thereby modulating their ability to successfully initiate BMP signaling in various ways [12]. The interaction of BMPs with ECM molecules depends on their distinct biochemical properties.

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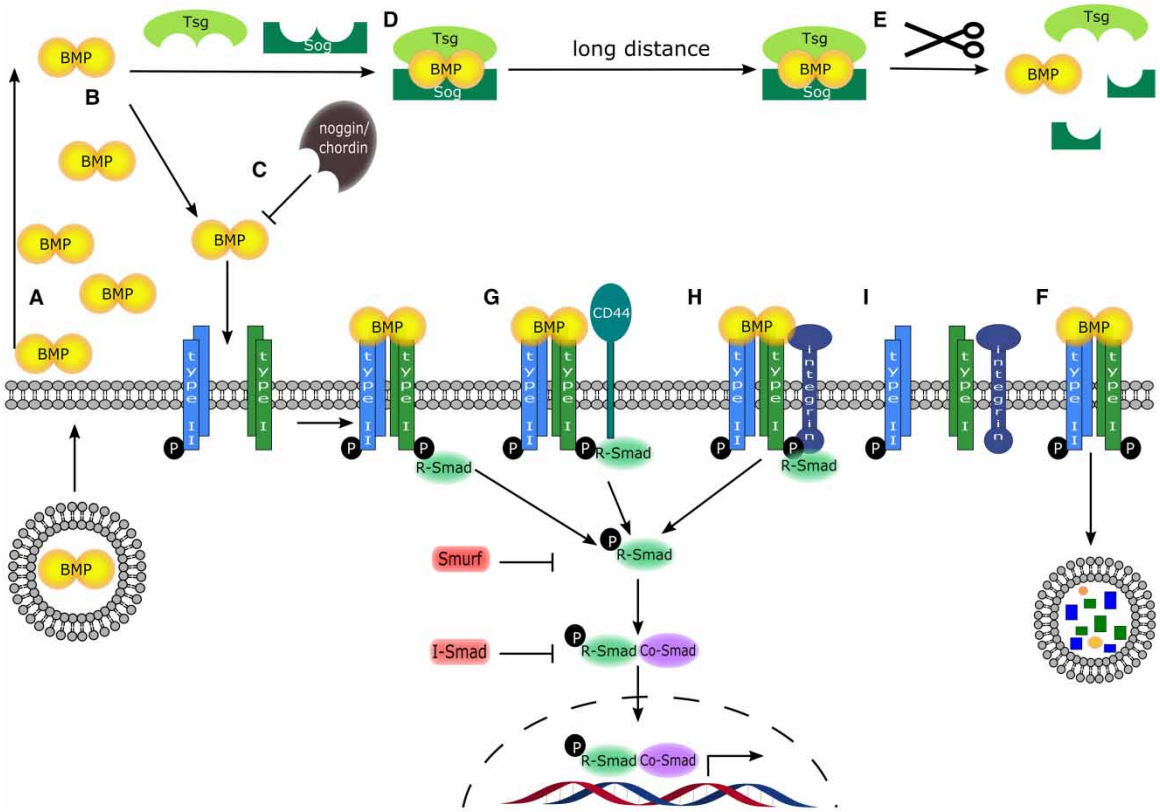

Figure 1. BMP signaling in the absence of regulation by ECM components.

(**A**) Following successful dimerization, BMP dimers are secreted and diffuse into the extracellular space. (**B**) BMP dimers bind to BMP type II receptors that subsequently heterodimerize and transphosphorylate BMP type I receptors. R-Smads become phosphorylated and form a complex with Co-Smads, which will be translocated to the nucleus to induce target gene expression. Smad signaling is intracellularly counteracted by I-Smads and Smurf proteins. (**C**) Extracellularly, BMP signaling is inhibited by BMP antagonisits, including noggin and chordin. (**D**) Ternary complex formation of Sog/Dpp/Tsg allows for long distance shuttling of Dpp. (**E**) Tolloid-mediated cleavage of Sog releases Dpp from the ternary complex and it is free to act at a distant site. (**F**) Receptor-mediated endocytosis of the BMP ligand/receptor complex. (**G**) CD44 binds Smad1 close to the cell membrane to facilitate Smad1 phosphorylation by BMPRs. (**H**) Integrins assist BMPR-mediated activation of R-Smads. (**I**) In the absence of extracellular signals, BMPRs and integrins do not engage without ligand binding.

ECM components bind to and sequester BMP signaling molecules and regulate their activity

In the early 1990s, it was discovered that BMPs bind with various degrees of affinity to several ECM components, including laminins and collagens [17]. Subsequent structural analyses revealed that the N-terminal prodomain of collagen type IIA binds BMP2 [18], whereas the C-terminal part of collagen type IV binds BMP4 [19].

Many ECM components are proteoglycans (PGs), proteins that are covalently linked to glycosaminoglycan (GAG) chains such as heparin, heparan sulfate, chondroitin sulfate (CS), dermatan sulfate (DS) and keratan sulfate. BMPs are capable of binding to heparins via a stretch of basic amino acid residues in the N-terminal region [20]. Negatively charged heparan sulfate proteoglycans (HSPGs), in particular, can bind to the N-terminal basic moiety of BMPs, [21] and it has been suggested that the degree of sulfation has an impact on the strength of the electrostatic interactions between HSPGs and BMPs [22]. However, binding of BMPs to HSPGs does not entirely depend on sulfated GAGs, as the protein core of the drosophila GPI-linked HSPG dally directly binds to BMP4 [23].

Small leucine-rich proteoglycans (SLRP) are ECM proteoglycans that consist of a protein core made up of leucine-rich repeats to which GAG chains of either CS or DS are attached [24]. Three members of the SLRP

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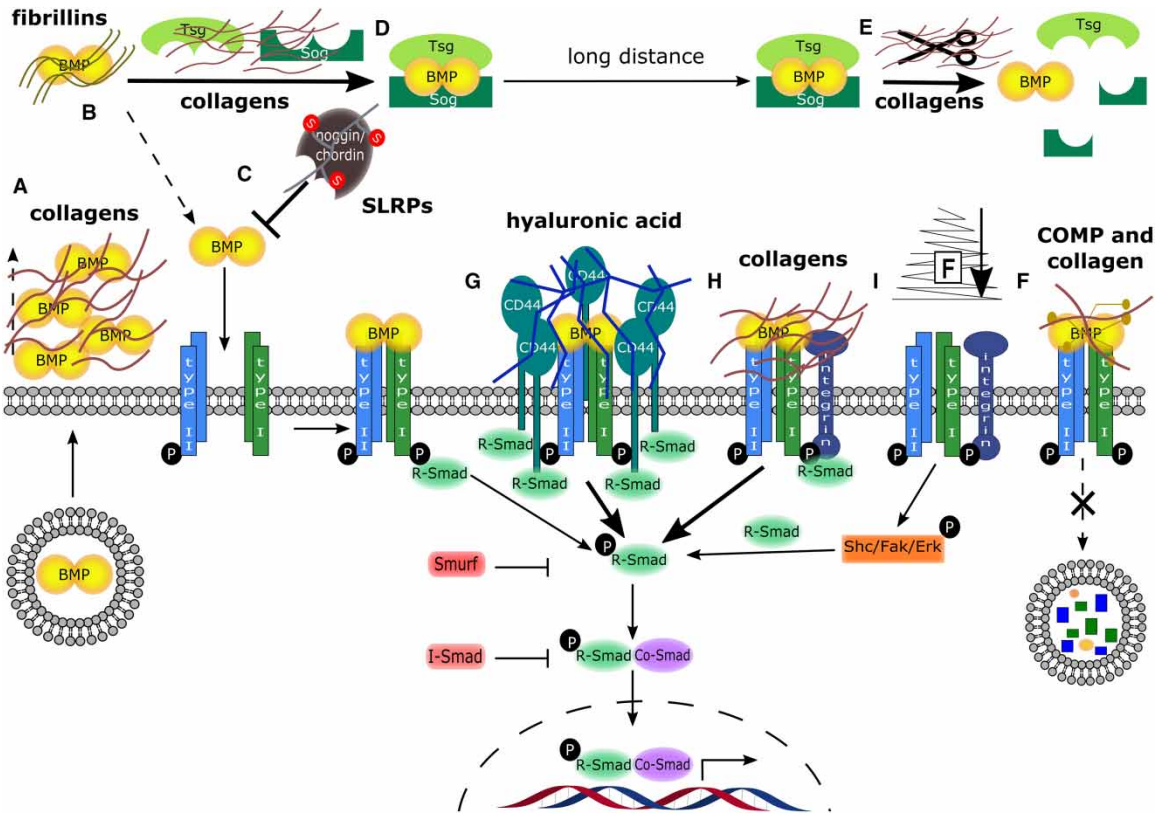

Figure 2. Regulation of BMP signaling by ECM components.

(**A**) ECM components inhibit the diffusion of BMP dimers into the extracellular space resulting in increased pericellular BMP levels that could result in enhanced autocrine BMP signaling. (**B**) Sequestration of BMPs by fibrillins prevents their binding to BMPRs. (**C**) SLRPs, such as biglycan and tsukushi, can bind to BMP antagonists and promote their inhibitory function of BMPs. (**D**) Collagen IV assists ternary complex formation of Sog/Dpp/Tsg by acting as a scaffold. (**E**) Sog cleavage by tolloid is assisted by collagen IV. (**F**) COMP prevents receptor-mediated endocytosis by bridging BMPs with collagens or proteoglycans. (**G**) Hyaluronic acid assists cluster formation of CD44, which binds Smad1 close to the cell membrane to facilitate its phosphorylation by BMPRs. (**H**) Collagen IV promotes the binding of integrins to BMPRs and thereby enhances BMP signaling. (**I**) Mechanic stress such as oscillatory shear stress or increased stiffness of the ECM [schematically represented as external force (F)] can induce complex formation of BMPRs with integrins, which induces Smad1 phosphorylation through Shc/Fak/Erk signaling in the absence of BMP ligands.

family, decorin, biglycan and tsukushi, can regulate BMP signaling extracellularly in different ways. All three SLRPs can exhibit inhibitory functions on BMP signaling. Decorin was demonstrated to inhibit BMP7-mediated signaling and thereby to antagonize nephron progenitor cell differentiation [25]. In addition, both decorin and biglycan have been shown to interfere with BMP2 activity [26]. BMP4 signaling can be inhibited by biglycan and tsukushi through the modulation of antagonist-mediated inhibition of BMP signaling. While biglycan promotes the binding of the BMP antagonist chordin to BMP4, which results in a more efficient inhibition of BMP4 signaling [27], the inhibition-promoting effect of tsukushi is due to its binding to both BMP4 and chordin, leading to ternary complexes of tsukushi, BMP4 and chordin, in which BMP4 signaling is co-operatively inhibited (Figures 1 and 2C) [28].

In contrast with the inhibitory function of biglycan on extracellular BMP signaling described above, other reports indicate a promoting function of biglycan on BMP signaling [29,30]. Biglycan was shown to have a promoting effect on BMP2 signaling *in vitro* in C2C12 myoblastic cells and *in vivo* in a rat mandibular defect model [29]. This promoting effect on BMP signaling was further enhanced by deglycanation (removal of GAG chains) of biglycan [29]. This suggests that GAG chains of biglycan are implicated in regulating the intensity of BMP2 signaling. In line with this finding, GAG chains of biglycan were found to be crucial for its stimulating

function on BMP4 signaling in murine calvarial cells [30]. In agreement with this observation, it was found that oversulfated CS, which is one of the main GAG chains of biglycan and other SLRPs, enhances BMP4-mediated osteogenesis of MC3T3-E1 osteoblastic cells [31]. However, another study found that oversulfated CSs themselves are capable of inducing osteogenic differentiation of human mesenchymal stromal cells independently of BMPs [32]. Therefore, the role of biglycan GAG chains in regulating BMP signaling activity needs further evaluation and is likely to be dependent on the cell type and BMPs investigated. In addition, more studies are needed to determine the role of GAG chains and their sulfation state in order to understand how extracellular proteoglycans, such as biglycan, bind to and interact with BMP signaling molecules.

Fibrillins are other major components of the ECM that bind to BMPs [33]. Fibrillins 1, 2 and 3 are self-assembling glycoproteins that aggregate to form microfibrils of 10–12 nm in diameter within the extracellular space, and which are critically involved in determining tissue elasticity and stiffness [34]. Binding of BMPs to fibrillins is mainly mediated by the BMP prodomain [35,36]. Prodomain binding to the N-terminal part of fibrillins can sequester BMPs within the ECM (Figures 1 and 2B) [35].

Sequestration of BMPs by the ECM within the extracellular space can inhibit binding to BMPRs. For example, binding of the BMP7 prodomain to fibrillin-1 causes a conformational change that prevents BMP7 from binding to its receptors [37]. However, BMPs can later be released through matrix remodeling, so that BMP sequestration serves a reservoir function [38]. Thus, the ECM regulates both the bioavailability and activity of BMPs secreted into the extracellular space.

ECM-assisted formation of multicomponent complexes that foster short- and long-range BMP signaling

A growing body of evidence indicates that BMP signaling is regulated by multicomponent complexes in which the ECM plays an instructive role by acting as a scaffold that directly shapes the intensity and duration of BMP signaling. For example, cartilage oligomeric matrix protein (COMP), an ECM glycoprotein, sustains BMP signaling by acting as a co-receptor that bridges BMPs with other ECM components such as collagen [39]. The authors suggest that COMP could assist complex formation of BMP2 with PGs or collagens, and thereby prevent endocytosis and degradation of BMP2/BMPR complexes (Figures 1 and 2F) [39]. Moreover, the ability of COMP to foster BMP signaling has been confirmed in an *in vivo* rat model of spinal fusion, where COMP was found to enhance bone-forming activity of low-dose recombinant BMP2 [40]. Structural analysis demonstrated that COMP binds directly to BMP2 via its C-terminal domain [41]. However, in this study, COMP was found to prevent BMP2 from binding to its receptors and thereby inhibit BMP2-mediated vascular smooth muscle calcification [41]. Therefore, the role of COMP in regulating BMP signaling appears to be context-dependent. Further studies are needed to determine whether COMP could be used for enhancing BMP signaling *in vivo*.

Another example is provided by hyaluronic acid (HA), a high molecular mass GAG that exists as a free polysaccharide in the ECM. The HA cell surface receptor CD44 interacts intracellularly with Smad1, which promotes BMP7 signaling responses (Figure 1G) [42]. Removal of pericellular HA from murine chondrocytes by hyaluronidase treatment was found to decrease BMP7-induced Smad1 phosphorylation [43]. HA-assisted BMP7 signaling is only effective at lower BMP7 concentrations and is not required when higher concentrations of BMP7 are present [43]. As HA is critical for CD44 clustering at the cell surface [44], HA could conceivably promote cluster formation of CD44/Smad1 close to the cell membrane and thereby facilitate BMPR-mediated phosphorylation of Smad1 (Figure 2G). Taken together, these findings suggest an instructive role for HA on BMP signaling that is mediated through CD44.

Other studies indicate that integrins can co-operate with BMPRs in an ECM-dependent manner, which augments BMP signaling. For example, the binding of the drosophila BMP2/4 homolog Decapentaplegic (Dpp) or human BMP4 to collagen type IV promotes BMP signaling in a manner that requires the binding of the collagen type IV to integrins [19,45]. Here, collagen acts as a scaffold that recruits integrins into a complex with the BMPR, which results in elevated levels of BMP signaling (Figures 1 and 2H). Integrin signaling is required for this effect. Other studies suggest that fibronectin-bound BMP2 can regulate a functional interaction between BMPRs and αvβ3 integrins [46]. In this study, αvβ3 integrins stimulated BMP signaling through fostering BMPR-induced Smad phosphorylation directly, as well as by suppressing GSK3 activity, leading to increased Smad stability.

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Within the extracellular space, several BMP-binding proteins form complexes with BMPs that increase their solubility and thus act as shuttles for BMPs to travel over longer distances [12]. In drosophila, the binding proteins short gastrulation (Sog) and twisted gastrulation (Tsg) form a soluble ternary complex with Dpp (Figure 1D) [47]. This complex formation can be facilitated by ECM components. Collagen IV was shown to act as a scaffold for ternary complex formation of Sog/Dpp/Tsg, in which Sog/Dpp is first complexed to collagen IV and the subsequent binding of Tsg releases the ternary complex Sog/Dpp/Tsg from collagen IV, so that it can freely move (Figure 2D) [48]. To act at a distant site, Dpp has to be released from the ternary complex. This release is mediated through tolloid metalloproteinase cleavage of Sog, which again is assisted by collagen IV that acts as a scaffold for the Sog–tolloid interaction (Figures 1 and 2E) [49].

Taken together, these examples demonstrate an important role for a variety of ECM components that act as scaffolds, which promote the formation of multiprotein complexes that regulate BMP signaling.

Physical properties of the ECM: BMP concentration gradients and mechanosignaling

Physical properties of the ECM include stiffness, elasticity and porosity, which can have an impact on BMP signaling, for example through the shaping of BMP concentration gradients. In addition, cells sense the mechanical properties of the ECM through mechanoreception, which can further regulate BMP signaling.

ECM-shaped concentration gradients of BMP molecules

Restricted diffusion in the ECM is critical for BMP gradient formation, for example during development [50]. These gradients can be determined in part through the biochemical interactions between ECM components and BMPs, as outline above. As an example, collagen IV immobilizes Dpp, whereas a Dpp mutant lacking collagen IV binding motifs diffuses over longer ranges *in vivo* [48]. However, in addition to inhibiting BMP diffusion through biochemical interactions, the ECM constitutes a mechanical barrier through which the diffusion of BMPs is hindered (Figures 1 and 2A) [15]. The diffusion coefficient of green fluorescent protein (GFP)–Dpp in the wing imaginal disc is $0.10 \pm 0.05\ \mu m^2/s$ [51], which is highly reduced compared with that of GFP in free solution (diffusion coefficient of $87\ \mu m^2/s$) [52]. Consistently, a secreted form of GFP fails to form a concentration gradient [50]. Several studies suggest a restricted diffusion of BMPs along ECM components such as HSPGs [53,54], which may be caused, at least in part, by transient reversible interactions between these ECM components and BMPs. ECM porosity may also regulate the diffusion rates of BMPs in different ECM contexts.

In addition to helping form gradients, reduced diffusion rates of secreted BMPs, in particular ECM contexts, could lead to local accumulation of BMPs in the ECM around cells that secrete BMPs. Increased pericellular levels of BMPs would, in turn, activate autocrine BMP signaling, if the producing cells also express the appropriate BMPRs. Thus, ECM microenvironments that reduce BMP diffusion would be predicted to sensitize cells to autocrine BMP signaling.

ECM-mediated mechanotransduced BMP signaling

Cells respond to the physical properties of the ECM surrounding them through mechanoreception. Smad1/5/8 phosphorylation is observed upon compressive strain in osteoblasts [55] and upon oscillatory shear stress (OSS) in endothelial cells independent of BMP ligand binding (Figures 1 and 2I) [56]. Furthermore, Smad1/5/8 phosphorylation upon OSS in endothelial cells depends on the interaction between BMPRIB and $\alpha_v\beta_3$ integrin, which form a mechanoreceptor complex that mediates Smad1/5/8 phosphorylation through the Shc/Fak/Erk pathway [57]. Bone marrow mesenchymal stem cells embedded in soft substrates exhibit increased β1 integrin-mediated internalization of BMPRIA compared with stiff substrates, which determines their differentiation fate [58]. Accordingly, BMPRI interacts with the integrin β1 subunit to induce Smad1/5 signaling in response to increased substrate stiffness [59]. Mechanical regulation of BMP signaling is also relevant *in vivo*, as mechanical load exhibited a stimulatory effect on BMP2-mediated bone healing in rats [60]. Thus, ECM stiffness directly regulates BMP signaling, with higher degrees of stiffness stabilizing BMPR/integrin complexes (Figure 2I). These findings further underscore the importance of integrins in regulating cellular response to BMPs, as integrin and BMP signaling converge to regulate cellular responses to the mechanical properties of the ECM.

Conclusion

BMP signaling clearly involves more than just BMPs and their receptors. Here, we have reviewed how the composition of the ECM and its biochemical and physical properties act in a variety of ways to positively or negatively regulate the activation of BMPRs, and show that the ECM is a dynamic and instructive regulator of BMP signaling. In addition to sequestering BMPs and their antagonists, ECM components act as important scaffolds that assist the formation of multiprotein complexes that regulate BMP signaling. A further level of complexity that has not been fully addressed here is the ability of a variety of cell surface molecules to act in *cis* and cross-regulate BMP signaling [12]. Clearly, the ECM is capable of regulating the function of some of these *cis*-acting surface proteins; for example, CD44 and specific integrins as outlined above. In future studies, it will be interesting to investigate whether the ECM also has an impact on the activity of co-receptors for BMP signaling such as repulsive guidance molecules [61], neogenin [62], betaglycan (TβRIII) [63] and endoglin (CD105) [64]. It will also be important to translate findings regarding the importance of ECM/BMP interactions made solely with cultured cells to the *in vivo* setting, where a complex ECM containing multiple components that can each regulate BMP signaling is present. Furthermore, many BMP antagonists bind to cell surface HSPGs via the GAG moiety [65,66]. We note that many ECM components are also HSPGs, so tethering of BMP antagonists to the ECM may add a further level of regulation. Thus, despite great advances in understanding the extracellular regulation of BMP signaling by the ECM, much remains to be discovered.

Abbreviations

BMP, bone morphogenetic protein; BMPR, bone morphogenetic protein receptor; COMP, cartilage oligomeric matrix protein; CS, chondroitin sulfate; Dpp, decapentaplegic; DS, dermatan sulfate; ECM, extracellular matrix; GAG, glycosaminoglycan; GFP, green fluorescent protein; GPI, glycosylphosphatidylinositol; HA, hyaluronic acid; HSPG, heparan sulfate proteoglycans; I-Smads, inhibitory Smads; OSS, oscillatory shear stress; PG, proteoglycan; SLRP, small leucine-rich proteoglycans; Smad, mothers against decapentaplegic; Sog, short gastrulation; TGF-β, transforming growth factor-β; Tsg, twisted gastrulation.

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Competing Interests

The Authors declare that there are no competing interests associated with the manuscript.

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