State-of-the-art of the Bone Morphogenetic Protein research field: 13th International BMP Conference, Dubrovnik 2022

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This article was submitted to RAD CASA - Medical Sciences as the original article

Conflict of Interest Statement: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Received: 25 October 2022
Accepted: 13 December 2022
Published: 21 December 2022

Citation:
Štoković N, Ivanjko N, Matić Jelić I, Milešević M, Rumenović V, Blažević V, Rogina L, Bastalić I, Erjavec I, Bordukalo Nikšić T, Kufner V, Adanić D, Perić M, Pećina M, Kuber Sampath T, Vukičević S. State-of-the-art of the Bone Morphogenetic Protein research field: 13th International BMP Conference, Dubrovnik 2022
RAD CASA - Medical Sciences. 553=60-61 (2022): 84-99
DOI: 10.21857/ypn4ocd309

Abstract:

The 13th International BMP Conference was held in October 2022 in Dubrovnik. The conference was attended by more than 240 participants from North America, Europe, Asia, and Australia who got an insight into the latest achievements in basic, translational, and clinical research of BMP molecules through 75 lectures categorized into several scientific sections. This review paper provides the most important novel findings on the structure, function, and signaling of BMPs, the role of BMPs in patterning and organoids as well as the role of BMP in metabolism. Moreover, we discussed the role of BMPs in various diseases including cancer pathogenesis, pulmonary arterial hypertension, and fibrodysplasia ossificans progressiva (FOP). Finally, we provided an overview of the new BMP-based therapies in regenerative medicine that are currently in different stages of preclinical and clinical trials.

Keywords: Bone Morphogenetic Proteins, TGFβ, Regenerative medicine, 13th International BMP Conference

Sažetak:

Nova dostignuća u području koštanih morfogenetskih proteina: 13. međunarodna BMP konferencija, Dubrovnik 2022

13. međunarodna BMP konferencija održana je u listopadu 2022. godine u Dubrovniku. Na konferenciji je sudjelovalo više od 240 sudionika iz Sjeverne Amerike, Europe, Azije i Australije koji su kroz 75 predavanja u različitim znanstvenim sekcijama dobili uvid u najnovija dostignuća u bazičnim, translacijskim i kliničkim istraživanjima BMP molekula. U ovom radu smo prikazali najvažnije nove spoznaje o strukturi, funkciji i signalizaciji BMP molekula, njihovoj ulozi u razvoju te metabolizmu. Također, raspravili smo ulogu BMP molekula u patogenezi različitih bolesti uključujući karcinome, plućnu hipertenziju i progresivnu osificirajuću fibrodisplaziju. Konačno, u radu smo dali

pregled novih terapija temeljenih na BMP molekulama u regenerativnoj medicini koje se trenutno nalaze u različitim fazama razvoja i kliničkih testiranja.
Ključne riječi: Koštani morfogenetski proteini, TGFβ, Regenerativna medicina, 13. međunarodna BMP konferencija

Introduction

Bone possesses a unique potential for repair and regeneration. The biological basis of this phenomenom was discovered in 1965 when Marshall R. Urist published that demineralized bone matrix when implanted under the skin in rodents induces heterotopic bone formation and proposed the term „Bone Morphogenetic Proteins“ (BMP) for this unique bone property (1, 2). BMPs have been subsequently purified by A. Hari Reddi and others to be cloned by John Wozney and colleagues (3). Moreover, in the past decades the biology of BMPs have been extensively studied and their role in the development and morphogenesis, metabolism, bone repair as well as their structure and intracellullar signalling have been elucidated. Today the BMP field ranges from basic research to translational and clinical studies with ultimate goal to bring BMP-based therapies to patients. Following the inaugural International BMP Conference organized by A. Hari Reddi in Baltimore in 1994, International BMP Conferences have been organized periodically in different international venues (USA, Japan, Croatia, Belgium, Germany) and became the main platform for exchange of knowledge and presentation of key discoveries. Moreover, to allow exchange of knowledge and keep the BMP community together during the COVID-19 pandemic the online BMP forum was established in 2020 by Petra Knaus and An Zwijsen. In the October 2022, 13th International BMP Conference was held in Dubrovnik, bringing this renowned event for the second time to Croatia (Figure 1). The Conference programm covered all areas in the BMP field with participations of world-leading experts in the BMP field who delivered keynote lectures as well as young scientists who were provided an opportunity to share and discuss their results (Figure 2). In the following sections we provide the state-of-the-art in the BMP field as presented at the Conference by 75 renowned BMP experts some of whom were awarded for their contribution to the BMP field from basic research to clinical applications (Table 1, Figures 3-4).

Figure 1. The 13th International Conference on Bone Morphogenetic Proteins took place from 8th to 12th October 2022 in Dubrovnik, Croatia.

Figure 2. Participants from world leading universities and pharmaceutical companies of the 13th International BMP Conference held in Dubrovnik.

Figure 3. (A) Carl-Henrik Heldin presented the EMBO Keynote lecture "TGF-β signaling and promotion of tumor progression". (B) A. Hari Reddi introduced the Peter ten Dijke's "Marshall R. Urist" lecture on "TGF-β family receptor signaling and disease", and (C) Eileen Shore's "Charles Huggins" lecture on "Heterotopic ossification, tissue identity, and the BMP signaling pathway". (D-E) A. Hari Reddi and Rick Derynk awarded young scientists for their contributions to the BMP related scientific research who presented their results in "New Voices in BMP Research" sections: (D) Tim Herpelinck, (E) Gonzalo Sanchez-Duffhues, (F) Nikola Štoković and (G) Johanna Bolander. (H) Round table "Transitional medicine and clinical study testing of novel drugs for rare diseases" chaired by Frank Luyten (KU Leuven, Belgium) and Hermann Oppermann (Genera Research, Zagreb, Croatia) with speakers Radan Spaventi and Katarina Oreskovic (Triadelta Partners ltd., Zagreb, Croatia), Susan Rhee (Regeneron, Tarrytown, US) and Jenn Lachey (Keros Therapeutics, Lexington, US). (I) Susan Rhee presented the lecture "Challenges in FOP clinical trials".

BMP from Structure to Function

The Bone Morphogenetic Proteins (BMPs) are the largest subgroup of the Transforming Growth Factor β (TGF-β) family of extracellular signaling proteins. All BMPs, like other TGFβ family members, are initially produced as a single chain containing a larger N-terminal prodomain and a smaller C-terminal mature signaling domain (4). BMP ligands undergo multiple processing and posttranslational modifications before forming homodimers of two identical subunits via an intermolecular disulfide bond through a cysteine knot (5). BMPs regulate embryonic patterning, the closely related Growth and Differentiation Factors (GDFs) regulate cartilage and skeletal development, while the Activins (Acts) and Inhibins (Inhs) regulate the release of pituitary hormones and others (6). Important differences exist among BMPs with regard to pathway mechanics and effects on cellular behavior. It has been shown that morphogen gradients specify cell fates during development with a classic example being the BMP gradient's conserved role in embryonic dorsal-ventral axis patterning (7). In addition, by comparing burst kinetics in cells receiving different levels of BMP signaling recent studies showed that BMP signaling controls burst frequency by regulating the promoter activation rate (8).

Activins are multifunctional secreted proteins that play critical roles in growth, differentiation and homeostasis in a wide variety of cell types. The activins bind to and signal through three type I receptors: ALK4, ALK5, and ALK7 (4). Anti-Müllerian Hormone (AMH) is a glycoprotein hormone which shares structural and mechanistic homology with signaling proteins of the TGFβ family. Recent advancements in the field have provided valuable insight into the molecular mechanisms of AMH signaling (9).

BMP Receptors, Co-receptors and Pro-domains

The TGF-β superfamily of growth and differentiation factors plays diverse role in embryonic development and adult tissue homeostasis. Moreover, it leads to a range of human diseases when misregulated (10, 11). In addition, dysregulation of TGFβ response and its downstream signaling pathways contribute to cancer initiation, progression and metastasis (12). The members of the TGFβ family signal through heteromeric complexes of type I and type II receptors which than activate members of

Table 1. Awarded Laureates at the 13th International BMP Conference

EMBO Keynote Lecture	Carl-Henrik Heldin
Marshall R. Urist Lecture	Peter ten Dijke
Charles Huggins Lecture	Eileen Shore
BMP Scientists Appreciation Award	A. Harri Reddi Petra Knaus An Zwijsen Slobodan Vukičević
Young Investigator Award	Tim Herpelinck Gonzalo Sanchez-Duffhues Nikola Štoković Johanna Bolander
Best Poster Award	Wouter Dheedene Martina Rossi Viktorija Rumenović

the SMAD family of signal transducers (10). Combinatorial interactions in the heteromeric receptor and SMAD complexes, receptor-interacting and SMAD-interacting proteins and also cooperation with sequence-specific transcription factors allow versatility and diversification of TGFβ family responses (13). The BMP receptors type I (BMPR1a, BMPR1b, ALK2) and the BMP receptor type II (BMPRII, ActRII and ActRIIB) (14) form homomeric and heteromeric complexes that exist in distinct membrane areas and are differently modulated by their ligands. Repulsive Guidance Molecules (RGMs) are known as key players in many fundamental processes such as cell migration, differentiation, iron homeostasis, apoptosis, as well as development and homeostasis of many tissues and organs (15, 16). On the other hand, three RGMs (RGMa, RGMb/DRAGON, and RGMc/hemojuvelin) have been linked to the pathogenesis of various disorders ranging from multiple sclerosis (MS) to cancer and juvenile hemochromatosis (JHH). RGMs are crucial activators of BMP signaling and bind to BMP ligands with high affinity (17, 18). Crystal structures of all human RGMs in complex with the BMP ligand BMP2 revealed a common mode of binding and identified N-RGM as the high-affinity interaction site for BMP ligands (19). A comparison between the structures of the RGM–BMP and BMP–BMP type I receptor ectodomain complexes showed that RGM and the BMP type I receptor ectodomain of BMP-R1A share the same binding site on the BMP ligand. Simultaneous binding of the BMP type I and type II receptors to the BMP ligand is an essential requirement for canonical, SMAD-dependent downstream signaling (17, 18).

Crosstalk Between SMAD 2/3 and SMAD 1/5/8 Signalling

SMAD proteins are the major effector molecules in TGF-β signaling pathway and the role of TGF-β signaling through SMAD2/3 in tumor suppression and cancer progression has been studied (11, 20), while the importance of SMAD1/5 signaling is less known. Recently, it was shown that TGF-β-induced SMAD1/5 phosphorylation requires members of two classes of type I receptor, TGFBR1 and ACVR1, which has a significant role in many TGF-β-induced transcriptomes, and TGF-β-induced EMT with SMAD1/5 signaling is being essential to

Figure 4. (A) The award of the Conference Scientific Committee to A. Hari Reddi for the establishment and contribution to BMP conferences was presented by Dora Adanić (Genera Research, Zagreb, Croatia). (B) A. Hari Reddi recognized Slobodan Vukicevic's contribution in organizing the BMP conference in Dubrovnik. (C) Petra Knaus and An Zwijsen founded the outstanding "BMP forum" as a primary platform for spreading novel developments in the BMP field which was highly appreciated in the opening ceremony. (D) A. Hari Reddi closed the conference and thanked the organizers and participants of the conference for their outstanding presentations and discussions. (E) Mihaela Peric lectured on "Translational aspects of rhBMP6-based drug development", and as a member of the committee for poster evaluation awarded (F) Martina Rossi for the best poster. (G) Members of the Laboratory for mineralized tissues and colaborators (From left to right: Mihaela Peric, Tatjana Bordukalo-Niksic, Vera Kufner, Ivona Matic Jelic, Nikola Stokovic, Natalia Ivanjko, Marina Milesevic, Viktorija Rumenovic, Valentina Blazevic, Ivancica Bastalic, Igor Erjavec, Lucija Rogina) with Slobodan Vukicevic and A. Hari Reddi.

induce ID1 (21). In addition, in ongoing research in several models of breast carcinoma, it has been shown that SMAD1/5 signaling is dynamically regulated at different steps of tumourigenesis hence inhibition of SMAD1/5 signaling pathway may represent a possible therapeutic target for the treatment of breast cancer (22, 23).
On the other hand, gain-of-function mutations in the cytoplasmic domain of type I BMP receptor, ACVR1, are causing FOP and Activin A, which normally antagonizes BMP signaling mediated through ACVR1, is the obligate physiological factor for the formation of heterotopic bone in FOP because FOP-mutant ACVR1 recognize Activin A as an BMP agonist (24). In contrast, wild-type ACVR1 forms non-signaling complexes (NSC) with Activin A and ties it down to type II receptors which render them inaccessible to BMPs which provides the first direct evidence for the role of non-signaling complexes in FOP (25). Recent results reveal an additional novel property of FOP-mutant ACVR1 which is activated by ACVR1 antibodies causing exacerbated rather than ameliorated heterotrophic ossification in mice. Conversely, wild-type ACVR1 is inhibited by ACVR1 antibodies which indicate that ACVR1 antibodies should not be considered as therapeutics for FOP (26).

Genomic Regulation by BMP Signalling

Structural research into Smad function led to recent advances in the structures of SMAD proteins using a multidisciplinary approach that combines X-Ray, NMR, MD simulations and SAXS data (27). In the past years, BMP research mostly focused on revealing intricate patterns of cellular communication led by BMP ligands and transcription factors. ZEB2 is a transcription factor that co-interprets nuclear BMP-Smad signaling in the BMP context, but also acts independent of Smads in differentiation and maturation of many different cell types, including those in chronic disease and acute injury (28, 29). ZEB2 binds to its own promoter and its role in the control of many monogenic developmental disorders classify this TF as a potential modifier (30). BMP signaling plays an important role in autoimmune disease induction and progression. Sjögren's disease (SjD) is a chronic autoimmune sialadenitis resulting in salivary gland hypofunction with dry mouth symptom (31). Salivary gland hypofunction was associated with decreased expression of sodium-potassium-chloride cotransporter-1 (NKCC1) and aquaporin 5 (AQP5) (32). Upregulated BMP6 expression was found in SjD patients and correlated with low unstimulated saliva flow rate. Furthermore, inhibition of BMP6 signaling, by reduction in SMAD 1/5/8 phosphorylation, led to recovery of submandibular gland function in the mouse model of SjD (33). The SjD development mechanism of action that was proposed, includes activation lysosome-associated membrane protein 3 (LAMP3) which in turn triggers the release of heat shock protein 70 (Hsp70) and BMP6 overexpression in salvary glands (34). Furthermore, LAMP3, as a pivotal molecule in SjD, initiates cell apoptosis and extracellular release of autoantigens leading to the formation of autoantibodies and exacerbation of the Sjögren's disease (35). While bone tissue response to mechanical stimulus is well researched, highlighting role BMP signaling in the process, the involvement of BMP signaling in vascularization is not sufficiently researched. Cardiovascular diseases are among the most prevalent pathological conditions in western world (36), and the involvement of BMP signaling in the disease formation warrants an in-depth research. The crosstalk of TGFβ/BMP signaling with mechanobiology in endothelium, that is constantly exposed to mechanical triggers, such as wall shear stress by blood flow or strain from the surrounding cells and tissue is not well understood. Fluid shear stress (FSS) in vasculature governs vessel *de novo* formation and remodeling to maintain physiological tissue structure (37). Abnormal patterns of FSS have been shown to enhance TGFβ/BMP signaling, where low FSS was found to enhance TGFβ/BMP signaling (38). Furthermore, low FSS enhanced endothelial SMAD1/5 signaling via the BMP9/ALK1 receptor complex that was further enhanced by Endoglin co-receptor. Since increased BMP signaling plays a role in endothelial-to-mesenchymal transition and inflammation, overstimulation of SMAD1/5 signaling can increases the risk of atherosclerosis (39). Additionally, low FSS was shown to facilitate the translocation of SMAD2/3 to the nucleus further enhancing TGFβ/BMP signaling (37).
To respond to mechanical stimulus the cytoskeleton plays a key role in the transduction of forces in endothelial cells. Mechanical forces influence the TGFβ/BMP pathway on multiple levels, such as type I and II receptors, intracellular SMADs and global cell-architecture and nuclear chromatin re-organization (40).

Bone: Diseases and Regeneration

Bone tissue is capable of regeneration and repair upon injury or fracture. On the other hand, various bone diseases largely increase skeletal fragility by disrupting the balance between bone formation and bone resorption. This delicate process is governed by osteoblasts (OBs) and osteoclasts (OCLs), which interact in diverse ways, as recently reported (41-43). Physical interaction between OBs and OCLs happens through cellular projections named tunneling nanotubes, which transport cargo towards OBs, leading to osteoblastic cell death in vitro (44). In a newly developed dual reporter model, OCLs are labeled using cathepsin-kCre (Ctsk-Cre) and OBs with osteocalcin-GFP which enables distinct observation between various phases of OCL-OB interaction. BMPs are important factors in signaling pathways during embryonic development in the migration, proliferation and differentiation of mesenchymal stem cells (45). Furthermore, BMPs are responsible for adult fracture healing by utilizing the same processes seen in embryonal bone formation. Their involvement in many aspects of bone development and bone regeneration make BMPs, as well as key players in Smad signaling pathways, the potential targets for novel therapeutic solutions. BMP2 was explored in certain medical indications (46, 47) and

its activity was shown to regulate important osteogenic processes (48, 49), including skeletal stem cell (SSC) niches. It has been recently shown that the loss of BMP2 in the periosteal stem cell niche impedes appositional bone formation, leading to spontaneous fracture. BMP2 is a primary participant in postnatal skeletal homeostasis through its regulation of SSC niches and BMP2 signaling is required for the differentiation of osteoprogenitor cells into the fully functioning osteoblasts (50). On the other hand, the role of BMP3 has never been fully elucidated (51-53) but recent discoveries presented at the Conference highlighted a novel role of BMP3 in enabling chondrocyte differentiation following decreased expression of BMP3 and ALK2.

BMPs in Cancer

BMPs exhibit dual role in cancer pathogenesis, acting as protumorigenic or antitumorigenic agents, which depends on the cancer type and stage, and additionally influence the genetic or epigenetic background of the patient (54-57). It has been shown that BMPs have tumor-suppressive role in carcinogenesis by inhibiting proliferation of gastric and breast cancer including prostate cancer cells, induce differentiation of glioma-initiating cells, and inhibit glioblastoma tumor formation (55, 57). BMPs also control the self-renewal and fate choices made by stem cells in several tissues (55). On the other side, their roles are correlated to various aspects of carcinogenesis, such as epithelial-mesenchymal transition (EMT), angiogenesis, and cancer stem cells (54). For example, BMPs enhance the motility and invasiveness of various types of cancer cells, such as breast and prostate cancer, including malignant melanoma cells (56-59). EMT is characteristic for embryonic development and adult pathogenesis (fibrosis or cancer) (59-62) and can be induced by TGF-β, BMPs and transcription factors, such as Snail (63, 64). EMT induced by TGF-β correlates with stemness and invasiveness, while mesenchymal-epithelial transition (MET) is induced by TGF-β withdrawal and correlates with metastatic colonization (65, 66). Additionaly, BMPs inhibit the process of EMT, contributing to the malignant progression of cancer at advanced stages (55).
The role of BMPs in cancerogenesis has been widely studied in colorectal cancers and brain tumors. In colorectal cancer (CRC), BMP signaling is generally viewed as strictly tumor-suppressive (67), while more recent studies suggest that they also exhibit oncogenic roles contributing to the progression of CRC by promoting invasiveness, EMT, and tumor volume (55, 57, 68-70). For example, role of BMP4 is highly correlated with CRC (68), and it has been shown that inhibition of BMP4 induces apoptosis of colorectal cancer cells (57) making it a potential therapeutic target. Furthermore, Gremlin-1, an important BMP antagonist plays a major role in several processes associated with cancer development, including proliferation, migration, invasion, and EMT (71). High levels of Gremlin-1 are associated with CMS4 stromal subtype of CRC and it predicts poor prognosis in patients (71). From the perspective of therapeutic use, Gremlin-1 might be a promising prognostic biomarker and therapeutic target in colorectal cancers (71, 72). Brain tumor development is regulated by BMP signaling and in most cases BMPs have a suppressive role (55). Diffuse intrinsic pontine gliomas (DIPG) are a subset of high-grade gliomas (HGG) resulting in a lethal childhood brainstem tumor that exhibits a very low survival rates (55, 73). DIPG is a result of genetic mutations (K27M) on the ACVRI/ALK2 gene locus encoding the serine/threonine kinase ALK2 resulting in single amino acid substitution - lysine to methionine (55, 73). Interestingly, these somatic ACVR1 mutations in DIPG are identical to germline mutations found in FOP (55, 74, 75). Many DIPG tumors are exploiting Cordin-like-1 (CHRDL1) capacity to hijack BMP ligands. It has been shown that BMP signaling epigenetically regulates CXXC5, a zinc finger protein, which turns DIPG tumor cells from "*prolonged-stem-cell-like*" state to differentiation, making CXXC5 a tumor suppressor and positive regulator of BMP signaling (73). Furthermore, BMPs suppress the tumorigenic function of human glioma-initiating cells (GIC) by inducing cell differentiation, cell cycle arrest, and apoptosis (74, 76, 77). For example, several studies suggest that BMP4 is expressed in low-grade gliomas and gliomas harboring IDH1 mutations, serving as a favorable prognostic marker in gliomas (74, 78-80).

Vascular and Cardiovascular BMP Signalling

The vascular system plays a critical role during embryonic development as well as in tissue homeostasis and repair (81-83). Angiogenesis as the formation of new blood vessels from pre-existing ones is a crucial process which occurs primarily during embryonic development and is almost absent in adulthood when the endothelium is quiescent. Two members of the BMP family, BMP9 and BMP10, have recently emerged as key growth factors for vascular quiescence (84-86). Genetic analysis revealed that mutations in BMP receptor ALK1 and BMPRII, both expressed on endothelial cells, have been directly linked to two rare vascular diseases: hereditary hemorrhagic telangiectasia (HHT), and pulmonary arterial hypertension (PAH), respectively (87). Endothelial cells proliferation, migration and tube formation are critical in the process of angiogenesis. Ongoing studies on the BMP9/BMP10/ALK1 impact on vascular quiescence revealed many new differentially phosphorylated proteins in response to BMP9 and BMP10 in endothelial cells, highlighting new signaling pathways and biological processes that enhance understanding of the role of BMP9 and BMP10 in vascular homeostasis and diseases.
Hereditary hemorrhagic telangiectasia is an inherited vascular disorder caused by mutations in the Endoglin, named HHT1 or ACVRL1 gene, named HHT2 leading to pathological angiogenesis and the formation of vascular malformations. It has been suggested that HHT mutations may be deleterious predominantly in endothelial cells with specific effects on the communication between pericytes and endothelial cells leading to vessel instability (88, 89).

Whilst mural cells will be recruited to the vessels, impaired TGF-β/BMP signaling in endothelial cells will result in poor attachment of the mural cells to the endothelium leading to defective TGF-β activation and subsequently poor mural cell differentiation. Recently it has been found that defective TGF-β1 signaling in mural cells is responsible for the structural defects of the vessel walls in HHT1 and that impaired cerebral blood flow could be detected by functional Ultrasound Localization Microscopy opening new insights for the identification of ultrasound markers to detect microvascular dysfunctions in patients with HHT.

Emerging roles of BMP signaling in vascular biology, especially in endothelial cells, have been recently reviewed (90). Endogenous BMP9 has been recognized as an important protective factor for the pulmonary vascular endothelium that is down-regulated during inflammation while an exogenous BMP9 offers a potential therapy to prevent increased pulmonary endothelial permeability in lung injury (91). Paradoxically, BMP9 ligand has been proposed as a treatment for pulmonary hypertension and vascular remodeling, while inhibition of BMP9/ALK1 signaling can also attenuate pulmonary hypertension and vascular remodeling under some circumstances which demonstrate the multifunctional and context-specific nature of endothelial BMP9/ALK1 signaling.

Another progressive complex disease, pulmonary arterial hypertension is characterized by extensive remodeling of the pulmonary circulation and leads to severe right-sided heart failure and death. As recently reviewed (92, 93), TGF-β superfamily signaling has emerged as a central player in the pathogenesis of PAH, although, it is still unclear how mutations of molecules involved in TGF-β/BMP signaling pathway affect the maintenance of the pulmonary vascular integrity. Connections between the TGF-β/BMP axis, PAH-predisposing gene mutations, disease pathology, and clinical manifestations, will increase our understanding of the PAH pathogenesis and offer new therapeutic targets.

Although BMPR2 is expressed in various tissues throughout the body, multiple studies indicate the pulmonary endothelium as the cell type that is most critically impacted by BMPR-II loss in PAH (94-96). The silencing of BMPR2 in human pulmonary artery endothelial cells (HPAECs) reproduces aberrant proliferation and impaired translational repression (96, 97). Although the molecular basis of these effects has focused primarily on signaling via the BMPR-II receptor, the ongoing work shows the potential contribution of alternative gene products, such as circular RNAs (circRNAs).

Furthermore, the cooperation of three different vascular systems, the systemic, the pulmonary and the lymphatic circulation, each of which is lined on the inside by a single layer of endothelial cells is essential to keep the body healthy. The role of BMP/SMAD signaling in lymphatic vessels is poorly known (98). Recent work shows that BMP/SMAD signaling is very regionalized within different lymphatic vessels in mice which suggests a role in lymphatic endothelium heterogeneity.

Misregulated BMP signaling has been shown to be involved in the pathogenesis of skeletal and cardiovascular disorders as well as cancer. Despite the recent advances in therapeutic interventions, cardiovascular diseases remain the largest health problem worldwide. Depending on the context and the repertoire of ligands and receptors involved, TGFβs, BMPs and activins can be beneficial or determinantal for cardiovascular function which highlight the cell- and context-specific nature of this pathway (99). An *in vitro* model studying PAH-induced cardiac fibrosis of the failing right ventricle using iPS-derived cardiac fibroblast from BMPR2-PAH patients and healthy donors is in process of development. The model shows that fibroblast activation induces fibrosis and hampers scar-free regeneration while ex vivo cardiac culture system modulating BMP10 signaling dampens the fibrotic response.

The progressive understanding of the mechanisms governing blood vessel formation has resulted in attempts to induce the process in the ischemic heart through the delivery of recombinant proteins, genes, and cells (100). The major issue for most biological drugs is their short half-life, particularly when delivered systemically, and the lack of an effective way for targeting the heart. Clinical data showing increased specific isoform of the bone morphogenetic protein 1 (BMP1.3) levels in the plasma of patients with myocardial infarction (MI) suggest that this isoform plays an important role in MI since its inhibition by a specific monoclonal antibody reduces the cardiac fibrosis (101). Initial studies in cardiac field as in many clinical fields suggest that mAbs could be the first biological drug to enter the clinical stage because of their long, safe, successful application, and the possibility of administration multiple times at a precise dose (102).

BMP in Patterning and Organoids

For embryonic implantation and development BMP signaling is fundamental for proper organ and organism patterning (103). TGF-β superfamily members are expressed in the placenta and regulate the process of placentation through the activation of several signaling pathways (104). Embryo implantation in endometrium during early pregnancy is promoted by estrogen (E2) that activates signaling pathway networks. TGFβ/BMP family signaling in the uterus was shown critical for establishing and maintaining pregnancy, while follistatin (FST), autocrine glycoprotein, regulates TGFβ family signaling by selective inhibition by ligand binding. In the absence of uterine FST, expression and signaling of activin B are up-regulated, while BMP signaling is attenuated (105). BMPs are shown to directly control endometrial receptivity via activin receptor type 2 A (ACVR2A) and SMAD1/5 signaling pathway (106, 107). BMP7 is also involved in receptivity of the endometrium, regulating blastocyst implantation via the endoglin pathway (108). Upon implantation, BMP2 was shown to promote trophoblast cell invasion (109). Furthermore, fetal trophoblasts replace maternal arteries by forming embryonic endothelium via epithelial-to-mesenchymal

transition (EMT) under the influence of SNAIL. TGFβ family govern this process by transient induction of SNAIL via ALK2/ALK5 receptors and SMAD1/5 phosphorylation (110). Progression of peritoneal endometriosis caused reduction in BMP6 and SMAD4 expression in cumulus cells that are important for folliculogenesis and fertility in general (111).

The intestine relies on homeostasis between functionality and regenerative capacity, where the TGF-β superfamily of proteins plays a pivotal role in regenerative processes in the intestine (112). In order to research intestine in vitro, intestinal organoids are formed, as a three-dimensional cultures that resemble key aspects of the epithelium of origin (113). The intestinal epithelium undergoes constant cell renewal but is exposed to a vast array of stresses ranging from digestion, exposure to bacteria, viruses, chemicals and inflammatory stimuli. In the intestine two most common epithelial cell types are absorptive enterocytes and mucous-secreting goblet cells. The BMP gradient along the villus axis is governing states of enterocyte and goblet cells thus regulating their maturity (114). The intestinal epithelium has a pivotal role in both immune initiation and effector stages, when dealing with viruses, bacteria, and helminths, in which the processes are coordinated by lymphocyte cytokines such as IFNγ, IL-13, and IL-22. During immune response in the gut, BMP pathway, which acts in a negative feedback loop on immune type 2-driven tuft cell hyperplasia, is induced by IL-13 (115).

BMP in Metabolism

Iron homeostasis is tightly regulated to provide adequate iron for essential biologic processes, but to limit the toxicity of iron excess which is associated with several common disorders including anemia, beta-thalassemia, and hereditary hemochromatosis (116, 117). In previous research, the critical role of the BMP-SMAD pathway in controlling hepcidin transcription in the liver has been revealed (118) and in ongoing research, the main focus is to unravel the precise molecular mechanisms of how BMP-SMAD signaling coordinates hepcidin production in response to body iron levels and the iron demand of erythropoietic cells to maintain systemic iron homeostasis, which may ultimately help to identify novel molecular targets for the treatment of iron-related disorders (117). On the other hand, a new monoclonal antibody (REGN7999) inhibits serine-protease TMPRSS6 activity and causes a negative regulation of hemojuvelin, a co-receptor for BMP and regulator of hepcidin expression, is a potential therapeutic option for beta-thalassemia and other iron overload disorders. Furthermore, a novel therapeutical approach in cancer cachexia treatment is focused on increasing BMP-SMAD signaling in the liver by bypassing the inhibitory effect of erythroferrone (ERFE), a BMP scavenger in the liver, and FKBP12, an intracellular inhibitor of BMP receptor (119, 120).

In addition, BMPs have been shown to affect the pathophysiological process of glucose metabolism (121-123) and in the pancreatic β cell line was demonstrated the opposite effect of BMP6 and serotonin on insulin secretion from β cells, as well as a stimulatory effect of BMP6 on serotonin secretion, which will be further explored in ongoing study in the BMP6 knockout mouse model. On the other hand, the role of Growth Differentiation Factor 3 (GDF3) in the adult mammalian biology is contentious, and in cultured cells it has been shown the dual biological roles of GDF3, where GDF3 inhibits signaling activity of multiple BMP proteins and at the same time has an expression profile similar to TGFβ1 (124).

Heterotopic Ossification and Fibrodysplasia Ossificans Progressiva (FOP)

FOP is a rare genetic disorder characterized by intermittent episodes of ectopic bone formation in soft connective tissues (125, 126). Moreover, FOP is accompanied with other skeletal abnormalities such as the malformation of the great toe. The cause of FOP is a mutation of Activin A receptor type 1 (ACVR1), also known as Activin receptor-like kinase 2 (ALK2). ACVR1/ALK2 is a transmembrane kinase receptor which transduces osteogenic signaling following ligand binding (127). FOP arises from missense mutation of ACVR1/ALK2 by altering Argine 206 to Histidine (R206H) (128). This gain of function mutation enables activin A to initiate canonical BMP signaling in fibro-adipogenic progenitors (128) resulting with abnormal endochondral bone formation at extraskeletal sites. Moreover, mutated receptors are hypersensitive to BMPs (129). Although FPO is an extremely rare disease it has devastating impact on the patients suffering from this disease. Therefore, substantial effort has been put into development of therapeutic solutions for FOP. The efficacy of potential drugs has been evaluated in preclinical animal models using $ACVR^{Q207D}$-transgenic mouse line and $ACVR^{R206H}$-knockin mouse (126). Saracatinib, a potent ALK2 kinase inhibitor (126) and Palovarotene, a retinoic acid receptor gamma agonist (130) were identified as potential candidates for treatment of FOP. On the other hand, anti-ACVR1 antibodies were excluded due to exacerbation of heterotopic ossification (26, 128). However, identification of novel therapeutic targets provides hope that FOP might be successfully treated by safe and effective drugs.

BMPs in Regenerative Medicine and Clinical Development

Pseudoarthrosis, segmental defects and degenerative diseases of the spine are among the most complex clinical condition affecting large percent of populations (131). The need for therapeutic solution for these conditions have been addressed by extensive evaluation of osteoinductive BMPs and their carriers in preclinical trials (132-136). These efforts have eventually resulted with introduction of two BMP-based osteoinductive devices (containing rhBMP2 and rhBMP7 delivered on collagen sponge) to clinical practice in indications such as anterior lumbar interbody fusion (ALIF), acute tibial fractures, and maxillofacial

reconstructions (137-139). However, these devices have been extensively used *off-label* which resulted in severe side effects such as heterotopic ossification, due to large BMP doses used (up to 12mg) and rapid BMP release from collagenous carrier (139-141). The occurrence of aforementioned side effects raised safety concerns and imminent need to develop device efficacious at relatively low BMP doses with low and sustained BMP release profile. The following requirements are met with the introduction of Osteogrow, a novel therapeutic solution comprised of rhBMP6 delivered within autologous blood coagulum (142, 143). To improve the biomechanical properties of Osteogrow implants might be supplemented with allograft bone (Osteogrow A), host bone (Osteogrow B) and synthetic ceramics (Osteogrow C) (144-146). Osteogrow family of products have been proven safe and efficacious in rat subcutaneous assay, rabbit segmental defect model as well as rabbit and sheep posterolateral spinal fusion (PLF) model (144, 145, 147-155). Moreover, Osteogrow has been evaluated in the phase 1 study in patients with distal radius fractures (156), in phase 2 in patients with high tibial osteotomy (157) and in phase 2 in patients treated by posterolateral lumbar interbody fusion for degenerative disc disease. Novel drug Osteogrow showed accelerated bone healing with no serious side effects and no detectable anti BMP antibodies in the peripheral blood (142). Aforementioned findings provide hope that safe and efficacious therapeutic solution for bone regeneration will be soon available in the clinics.

Osteoarthritis (OA) is a complex clinical condition characterized by penetration of joint surface defects into underlying bone. Currently, the gold standard for treatment of patients with OA is osteochondral autograft transplantation. However, the long-term outcome in these patients is poor and there is an unmet clinical need for new therapeutic solutions in these indications (158). The role of BMPs in various cartilage explant and in animal models have been extensively explored (159, 160) but little progress towards clinical testing has been achieved. To address this imminent need, recently extensive preclinical studies have been conducted and different therapeutic solutions ranging from bilayered constructs with a stable cartilage patch on top of subchondral bone forming device to more complex cell based implants have been evaluated (158) with very promising outcome.

Fibrosis is a pathological process characterized by excessive deposition of extracellular matrix and replacement of parenchyme in critical clinical conditions such as chronic kidney disease, liver cirrhosis and myocardial infarction. BMP1.3 antibodies have been recently proposed as a therapeutic solution for treatment of renal, liver and cardiac fibrosis and evaluated in relevant animal models (101, 161, 162). Systemic administration of monoclonal BMP1.3 antibodies reduced collagen deposition and fibrosis and preserved physiological parenchyma suggesting that antibodies targeting BMP1.3 pathway might be therapeutic target in patients with aforementioned conditions (101, 161, 162).

Sotatercept, IIA ligand trap, has been evaluated in patients with pulmonary arterial hypertension. KER-012 is an investigational modified ActRIIB ligand trap designed to bind and inhibit activins to promote bone mass and regulate vascular homeostasis. In preclinical studies, a research form of KER-012 increased bone mass in healthy and osteoporotic mice and prevented vascular remodeling and cardiac damage. In clinical study, the safety and tolerability of escalating doses of KER-012 administered as single and multiple subcutaneous doses is under evaluation in Phase 1 in healthy postmenopausal women (163). Moreover, anabolic effects on muscle mass of wild-type ActRIIB-Fc and the novel ActRII-Fc ligand trap KER-065 has been demonstrated. KER-065 was observed to bind negative muscle regulators with reduced BMP9 inhibition and had a robust effect on muscle, even when compared to ActRIIB-Fc (164).

Conclusions

Exceptional lectures and findings presented at the 13th International BMP Conference summarized in this review proved that BMP research is extremely dynamic in both basic and translational aspects. We are especially excited about the development and clinical evaluation of novel BMP-based therapies which might be safe and efficacious therapeutic solutions for a broad range of clinical indications and improve the well-being of patients worldwide. Therefore, we are looking forward to further progress in this field and to the next, 14th International BMP Conference that will take place in the United States in 2024.

Acknowledgements

We are grateful for the support received for the organization of the 13th International BMP Conference from the following private corporates, and organizations: Regeneron Pharmaceuticals, Keros Therapeutics, Genera Research/perForm Biologics, Croatian Academy of Sciences and Arts, University of Zagreb, Ministry of Science and Education of the Republic of Croatia and Center of Excellence for Reproductive and Regenerative Medicine (University of Zagreb School of Medicine). We thank to Filida Travel Agency, Quam-quam and Valamar Lacroma hotel staff for their help in the technical organization of the Conference.

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