



FULL LENGTH ARTICLE

Highly expressed BMP9/GDF2 in postnatal mouse liver and lungs may account for its pleiotropic effects on stem cell differentiation, angiogenesis, tumor growth and metabolism

Wei Liu ^{a,b}, Zhongliang Deng ^{b,c}, Zongyue Zeng ^{b,d},
Jiaming Fan ^{b,d}, Yixiao Feng ^{a,b}, Xi Wang ^{b,d}, Daigui Cao ^{b,c,e},
Bo Zhang ^{b,f}, Lijuan Yang ^{b,f}, Bin Liu ^{b,g}, Mikhail Pakvasa ^b,
William Wagstaff ^b, Xiaoxing Wu ^{a,b}, Huaxiu Luo ^{b,h},
Jing Zhang ^{a,b}, Meng Zhang ^{b,i}, Fang He ^{a,b}, Yukun Mao ^{b,j},
Huiming Ding ^{b,k}, Yongtao Zhang ^{b,l}, Changchun Niu ^{b,e},
Rex C. Haydon ^b, Hue H. Luu ^b, Jennifer Moriatis Wolf ^b,
Michael J. Lee ^b, Wei Huang ^a, Tong-Chuan He ^b, Yulong Zou ^{b,c,*}

^a Departments of Orthopedic Surgery, Breast Surgery, Gastrointestinal Surgery, Obstetrics and Gynecology, and Nephrology, The First Affiliated Hospital of Chongqing Medical University, Chongqing 400016, China

^b Molecular Oncology Laboratory, Department of Orthopaedic Surgery and Rehabilitation Medicine, The University of Chicago Medical Center, Chicago, IL 60637, USA

^c Department of Orthopaedic Surgery, The Second Affiliated Hospital of Chongqing Medical University, Chongqing 400010, China

^d Ministry of Education Key Laboratory of Diagnostic Medicine, The School of Laboratory Medicine, Chongqing Medical University, Chongqing 400016, China

^e Departments of Orthopaedic Surgery and Laboratory Diagnostic Medicine, Chongqing General Hospital, Chongqing 400021, China

^f Key Laboratory of Orthopaedic Surgery of Gansu Province, The Departments of Orthopaedic Surgery and Obstetrics and Gynecology, The First and Second Hospitals of Lanzhou University, Lanzhou, 730030, China

^g School of Life Sciences, Southwest University, Chongqing 400715, China

^h Department of Burn and Plastic Surgery, West China Hospital of Sichuan University, Chengdu 610041, China

* Corresponding author. Department of Orthopaedic Surgery, The Second Affiliated Hospital, Chongqing Medical University, Chongqing 400010, China. Fax: +011 86 23 6382 2194.

E-mail address: rocketsoul@163.com (Y. Zou)

Peer review under responsibility of Chongqing Medical University.

<https://doi.org/10.1016/j.gendis.2019.08.003>

2352-3042/Copyright © 2019, Chongqing Medical University. Production and hosting by Elsevier B.V. This is an open access article under the CC BY-NC-ND license (<http://creativecommons.org/licenses/by-nc-nd/4.0/>).

ⁱ Department of Orthopaedic Surgery, The First Affiliated Hospital of Guangzhou University of Chinese Medicine, Guangzhou 510405, China

^j Department of Orthopaedic Surgery, The Affiliated Zhongnan Hospital of Wuhan University, Wuhan 430072, China

^k Department of Orthopaedic Surgery, BenQ Medical Center Affiliated with Nanjing Medical University, Nanjing 210000, China

^l Department of Orthopaedic Surgery, The Affiliated Hospital of Qingdao University, Qingdao 266061, China

Received 6 August 2019; received in revised form 22 August 2019; accepted 31 August 2019

Available online 14 September 2019

KEYWORDS

BMP9/GDF2;
Bone morphogenetic proteins (BMPs);
Hepatic metabolism;
Mesenchymal stem cells;
Neurogenesis;
Osteogenic differentiation;
Pulmonary arterial hypertension;
Tumorigenesis

Abstract Bone morphogenetic protein 9 (BMP9) (or GDF2) was originally identified from fetal mouse liver cDNA libraries. Emerging evidence indicates BMP9 exerts diverse and pleiotropic functions during postnatal development and in maintaining tissue homeostasis. However, the expression landscape of BMP9 signaling during development and/or in adult tissues remains to be analyzed. Here, we conducted a comprehensive analysis of the expression landscape of BMP9 and its signaling mediators in postnatal mice. By analyzing mouse ENCODE transcriptome datasets we found Bmp9 was highly expressed in the liver and detectable in embryonic brain, adult lungs and adult placenta. We next conducted a comprehensive qPCR analysis of RNAs isolated from major mouse tissues/organs at various ages. We found that Bmp9 was highly expressed in the liver and lung tissues of young adult mice, but decreased in older mice. Interestingly, Bmp9 was only expressed at low to modest levels in developing bones. BMP9-associated TGF β /BMP type I receptor Alk1 was highly expressed in the adult lungs. Furthermore, the feedback inhibitor Smads Smad6 and Smad7 were widely expressed in mouse postnatal tissues. However, the BMP signaling antagonist noggin was highly expressed in fat and heart in the older age groups, as well as in kidney, liver and lungs in a biphasic fashion. Thus, our findings indicate that the circulating BMP9 produced in liver and lungs may account for its pleiotropic effects on postnatal tissues/organs although possible roles of BMP9 signaling in liver and lungs remain to be fully understood.

Copyright © 2019, Chongqing Medical University. Production and hosting by Elsevier B.V. This is an open access article under the CC BY-NC-ND license (<http://creativecommons.org/licenses/by-nc-nd/4.0/>).

Introduction

Bone morphogenetic proteins (BMPs) are members of the TGF- β superfamily and play important roles in embryogenesis, organogenesis, cell proliferation, stem cell differentiation, and adult tissue homeostasis.^{1–10} Disruptions in BMP signaling cause skeletal and extraskeletal anomalies.^{1,4,11} At least 14 types of BMPs in humans and rodents have been identified.^{1,5,9,12,13} BMPs were initially discovered when demineralized bone was found to induce *de novo* bone formation.^{14,15}

Different BMPs exert distinct but overlapping biological functions. BMP9 (also known as growth differentiation factor 2 or GDF-2) represents a less characterized member of the BMP family.^{4,9,13} Through a comprehensive analysis of human BMPs in mesenchymal stem cells (MSCs), we demonstrated that BMP9 is one of the most potent BMPs among the 14 types of BMPs in inducing osteogenic/odontogenic differentiation of MSCs.^{9,10,16–19} We also found that BMP9 is resistant to the naturally occurring antagonist noggin,²⁰ and demonstrated that TGF β /BMP type I receptors ALK1 and ALK2 are essential for BMP9-induced osteogenic signaling in MSCs.²¹ As BMP9 is one of the least studied BMP, we performed mechanism-based studies and

identified several early downstream targets,^{9,10,22–28} and demonstrated that BMP9 signaling has extensive cross-talks with other signaling pathways, especially Wnt and Notch signaling.^{9,28–37}

It has also been reported that BMP9 plays important roles in inducing and maintaining basal forebrain cholinergic neurons, inhibiting hepatic glucose production, inducing the expression of key enzymes of lipid metabolism, stimulating hepcidin 1 expression, regulating angiogenesis, and modulating tumorigenesis.^{10,38} BMP9 has further been implicated in the pathogenesis of pulmonary arterial hypertension.^{38,39}

Originally identified from fetal mouse liver cDNA libraries, BMP9 is highly expressed in the developing mouse liver.^{4,9,40} Increasing evidence indicates that BMP9 exerts diverse and pleiotropic biological functions during postnatal development and in maintaining adult tissue homeostasis. Thus, it is conceivable that a postnatal expression landscape of BMP9 and its important signaling mediators would provide important insights into the potential functions of BMP9 signaling in major organs and/or tissues. However, the expression landscape of BMP9 and its signaling mediators during development and/or in adult tissues has yet to be fully investigated.

In this study, we sought to conduct a comprehensive analysis of the expression landscape of BMP9 and its signaling mediators in postnatal mice. We first analyzed the mouse ENCODE transcriptome data sets and found that *Bmp9* was highly expressed in liver although *Bmp9* expression was detectable in embryonic brain, adult lungs and adult placenta. However, the ENCODE datasets are limited in scope and depth. Thus, we conducted a comprehensive qPCR analysis of the RNAs isolated from major mouse tissues/organs at various ages. Our results demonstrated that, although at relatively lower levels at birth, *Bmp9* was highly expressed in the liver and lung tissues of young adult mice, but decreased in older mice. *Bmp9* expression was also detectable in 2-week spleen, brain and fat tissues and muscle samples. Interestingly, *Bmp9* was only expressed at modest to low levels in developing bones. *Alk1* was highly expressed in the adult lungs, while *Alk2* was expressed at relatively higher levels in fat and kidney tissues. Furthermore, the feedback inhibitors *Smad6* and *Smad7* of BMP signaling were widely expressed in mouse postnatal tissues, including developing bones. However, the BMP signaling antagonist *noggin* was expressed highly in fat and heart tissues in the older age groups, as well as in kidney, liver and lung tissues in a biphasic fashion. Collectively, our findings indicate that the circulating BMP9 produced in liver and lungs may account for its pleiotropic effects on multiple postnatal tissues/organs although the functional roles of BMP9 signaling in liver and lungs remain to be fully understood.

Materials and methods

Mouse ENCODE transcriptome data analysis

The RNA profiling data sets were generated by the Mouse ENCODE project PRJNA66167 as described.⁴¹ The data sets included 30 mouse samples derived from embryonic and adult tissues, and are publically available through NCBI website (<https://www.ncbi.nlm.nih.gov/gene/>). The acquired reads per kilobase per million reads (RPKM) for *Bmp9*, *Alk1*, *Alk2*, *Smad6* and *Smad7* in the 30 mouse samples were graphed by using the Microsoft Excel software.

Animal tissue samples

The use of mouse tissues was approved by the Institutional Animal Care and Use Committee and all experimental

procedures involved in harvesting mouse tissues were performed according to the approved guidelines. Briefly, C57BL/6J mice at newborn (NB), 2-week, 4-week, 3-month, 8-month and 18-month old (male, $n = 5$) were obtained from The University of Chicago Transgenic Core Facility.

Total RNA isolation from mouse tissues and reverse transcription

For total RNA isolation, mouse brain, fat (inguinal region), heart, kidney, liver, lung, muscle, spleen, femur and parietal bone (PB) at various ages were harvested immediately after sacrificing the animals, placed in RNase-free mortars containing NucleoZOL Reagent (Takara Bio USA, Mountain View, CA) and liquid nitrogen, and crashed with RNase-free pestles in a RNase-free biosafety cabinet. Total RNA was subsequently isolated according to the manufacturer's introduction, and subjected to reverse transcription with hexamer and M-MuLV (New England Biolabs, Ipswich, MA). The cDNA products were diluted as templates for qPCR.

Touchdown quantitative real-time PCR (TqPCR)

The qPCR primers were designed by using Primer3 Plus program.⁴² SYBR Green-based quantitative real-time PCR analysis was performed by following our previously optimized TqPCR protocol.⁴³ The qPCR reactions were done in triplicate. All expression values were normalized to the reference gene *Gapdh* expression by using the $2^{-\Delta\Delta C_t}$ method.^{44–47} The qPCR primer sequences are listed in Table 1.

Results and discussion

Mouse ENCODE transcriptome data indicate *Bmp9* is highly expressed in liver while *Bmp9*-associated BMP type I receptor *Alk1* is highly expressed in lungs

As one of the least studied BMPs, BMP9 was originally identified from mouse embryonic liver tissue.⁴⁰ However, the expression patterns of BMP9 and its signaling mediators during development and/or in adult tissues have not been investigated. The recently completed ENCODE transcriptome projects have offered a glimpse of the expression patterns for a given gene.

Table 1 List of qPCR Primers.

Gene	Accession No.	Forward	Reverse
<i>Bmp9/Gdf2</i>	NM_019506.4	TGAGTCCCATCTCCATCCTC	ACCCACCAGACACAAGAAGG
<i>Alk1</i>	NM_001277255.1	ACCTGGGACTGGCTGTGA	GCAGTCTGTGCGGATGTG
<i>Alk2</i>	NM_001110204.1	GTGGCTCCGGTCTTCCTT	AGCGACATTTTCGCCTTG
<i>Smad6</i>	NM_008542.3	ATCACCTCCTGCCCTGT	CTGGGGTGGTGCTCTGG
<i>Smad7</i>	NM_001042660.1	AAGATCGGCTGTGGCATC	CCAACAGCGTCCTGGAGT
<i>Nogging</i>	NM_008711.2	GCGGCCAGCACTATCTACA	GGGGCGAAGTAGCCATAAA
<i>Gapdh</i>	NM_008084	GAAGGTCGGTGTGAACGGAT	ACTGTGCCGTTGAATTGGCC

Here, we took advantage of the mouse ENCODE database and examined the expression patterns of *Bmp9* and its signaling mediators in 30 mouse samples. Consistent with earlier reports,⁴⁰ *Bmp9* expression level was apparently high in liver E14, E14.5, E18 and adult liver samples, while *Bmp9* expression was also detected in CNS E11.5, adult lung, and adult placenta (Fig. 1A). Surprisingly, *Bmp9* associated BMP type I receptor *Alk1* was highly expressed in adult lung tissue although several tissues (such as adult bladder, colon, genital fat pad, heart, kidney, mammary gland, ovary, and subcutaneous fat pad) expressed detectable levels of *Bmp9* (Fig. 1B). Conversely, another *Bmp9*-associated type I receptor *Alk2* exhibited a broad range, even though many of those tissues exhibited medium to lower levels of *Bmp9* expression (Fig. 1C).

We also analyzed the ENCODE data for BMP signaling feedback inhibitor Smads (or I-Smads), *Smad6* and *Smad7*. Surprisingly, adult lung tissue has the highest expression for

both I-Smads, followed by various GI tissues, although *Smad7* is more widely expressed than *Smad6* (Fig. 1D & E). Taken together, the mouse ENCODE transcriptome data indicate that *Bmp9* expression is relatively restricted to liver, and to a lesser extent, lungs, while the *Bmp9* signaling mediators, especially *Alk1*, *Smad6* and *Smad7*, exhibit high expression levels in adult lungs, suggesting that BMP9 may play an important role in regulating biological functions and tissue homeostasis of the lungs.

Bmp9 is highly expressed in postnatal liver and lung tissues

Even though the ENCODE database has offered a quick overview of the expression of *Bmp9* and its signaling mediators, those data are limited in scope and depth. On the other hand, the currently available antibodies against BMP9

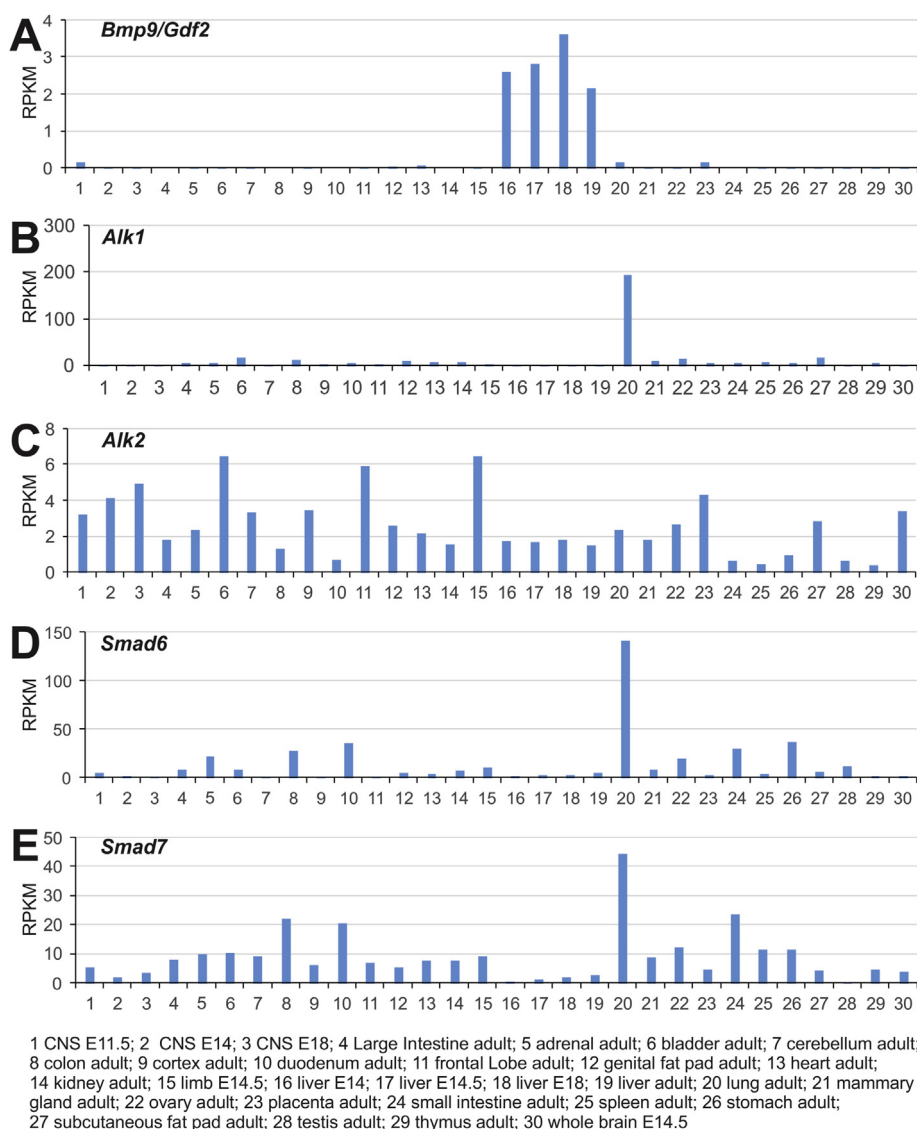


Figure 1 Across-tissue expression of *Bmp9* and its signaling mediators revealed by mouse ENCODE transcriptome analysis. The RNA profiling data sets ($n = 30$ samples) were generated by the Mouse ENCODE project, PRJNA66167, as described in.⁴¹ The tabulated reads per kilobase per million reads (RPKM) for *Bmp9* (A), *Alk1* (B), *Alk2* (C), *Smad6* (D) and *Smad7* (E) in the 30 mouse samples were graphed. The individual samples were delineated at the bottom of the graphs.

lack either specificity and/or reactivity. Furthermore, it is relatively difficult to quantify immunostaining results. Thus, we decided to carry out qPCR analysis of the postnatal expression of *Bmp9* and related signaling mediators. We collected total RNA from the major mouse tissues/organs, such as whole brain, fat (inguinal region), heart, kidney, liver, lungs, muscle, spleen, femur, and parietal bone (PB) at various ages, including newborn (NB), 2-week, 4-week, 3-month, 8-month and 18-month old mice.

Using our previously optimized touchdown qPCR (or TqPCR) technique,⁴³ we analyzed the expression of *Bmp9* in these tissues. At age groups, *Bmp9* was shown to express at much higher levels in liver and lung tissues, although its expression showed an age-dependent trend in decrease (Fig. 2A). Interestingly, a significant expression peak of *Bmp9* was found in 2-week spleen tissue (Fig. 2A). A detailed analysis indicates that *Bmp9* expression was readily detectable in 2-week brain, 2-week and 8-month fat tissue, and in muscle at most of the analyzed time points (Fig. 2B, panels a, b, and e). Interestingly, even though highly expressed at later time points (e.g., 2-week, 4-week and 3-month), *Bmp9* was expressed at relatively low levels in both liver and lung tissues at birth (Fig. 2B, panels c & d). In bone tissue such as femur, *Bmp9* expression increased over the time from newborn to 3-month (Fig. 2B, panel f),

indicating BMP9 may play an important role in bone and skeletal development.

Bmp9-associated TGF β /BMPR type I receptor *Alk1* is highly expressed in the lungs, while *Alk2* expressed relatively higher in fat and kidney tissues

We previously demonstrated that BMP9 initiates its signaling through interactions with TGF β /BMPR type I receptor *Alk1* and *Alk2*²¹. Our results indicated that *Alk1* was modestly expressed in various age groups of the brain, heart, kidney and liver tissues (Fig. 3A, panels a, c, d, and e). However, the highest expression levels of *Alk1* were found in the lungs, especially in 3-month lungs, although *Bmp9* was also highly expressed in 8-month fat tissue (Fig. 3A, panels c & f). On the other hand, *Alk2* was expressed modestly, but more broadly in the analyzed tissues, including brain, fat, heart, kidney, liver and lungs, although the highest level of *Alk2* expression was found in 8-week fat tissue sample (Fig. 3B, panels a to f).

Lastly, we examined the expression of *Alk1* and *Alk2* in the developing bone tissues, femurs and parietal bone (PB). We found that the expression of *Alk1* and *Alk2* peaked at the 4-week time point in both bone samples (Fig. 3C, panels

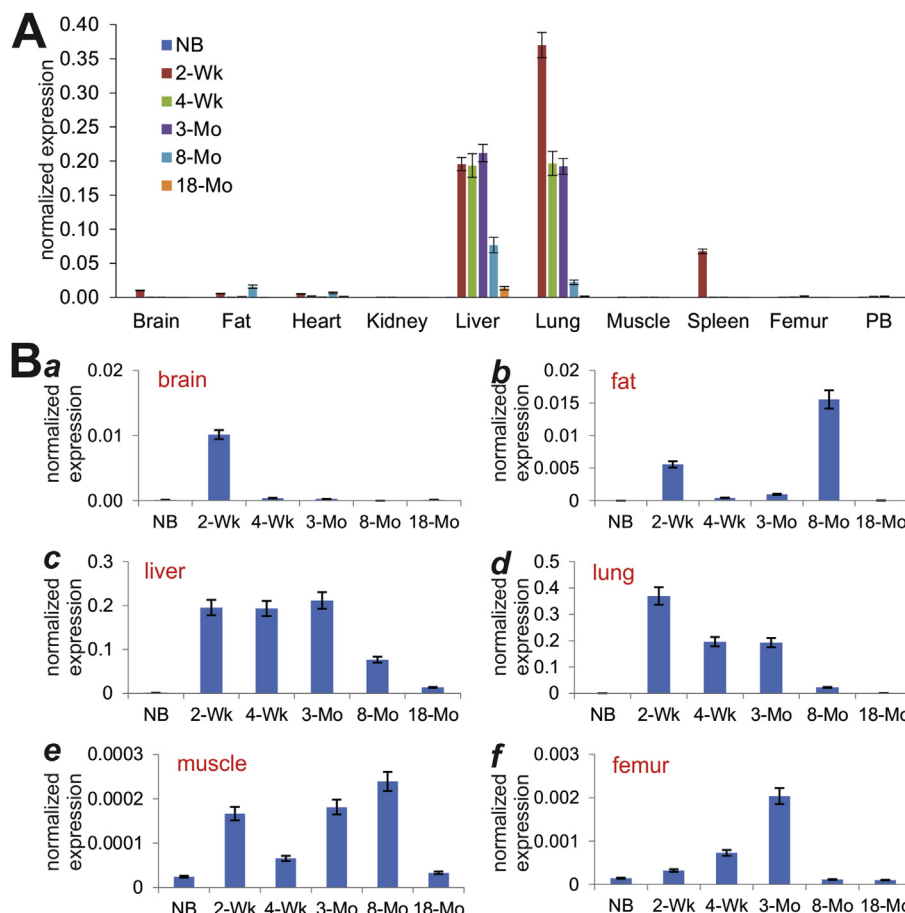


Figure 2 Expression of *Bmp9* in postnatal mouse tissues determined by TqPCR analysis. Total RNA was isolated from mouse brain, fat (inguinal region), heart, kidney, liver, lung, muscle, spleen, femur, and parietal bone (PB) at various ages: newborn (NB), 2-week, 4-week, 3-month, 8-month and 18-month old. The RT cDNA products were subjected to TqPCR analysis of *Bmp9* expression in these tissues.

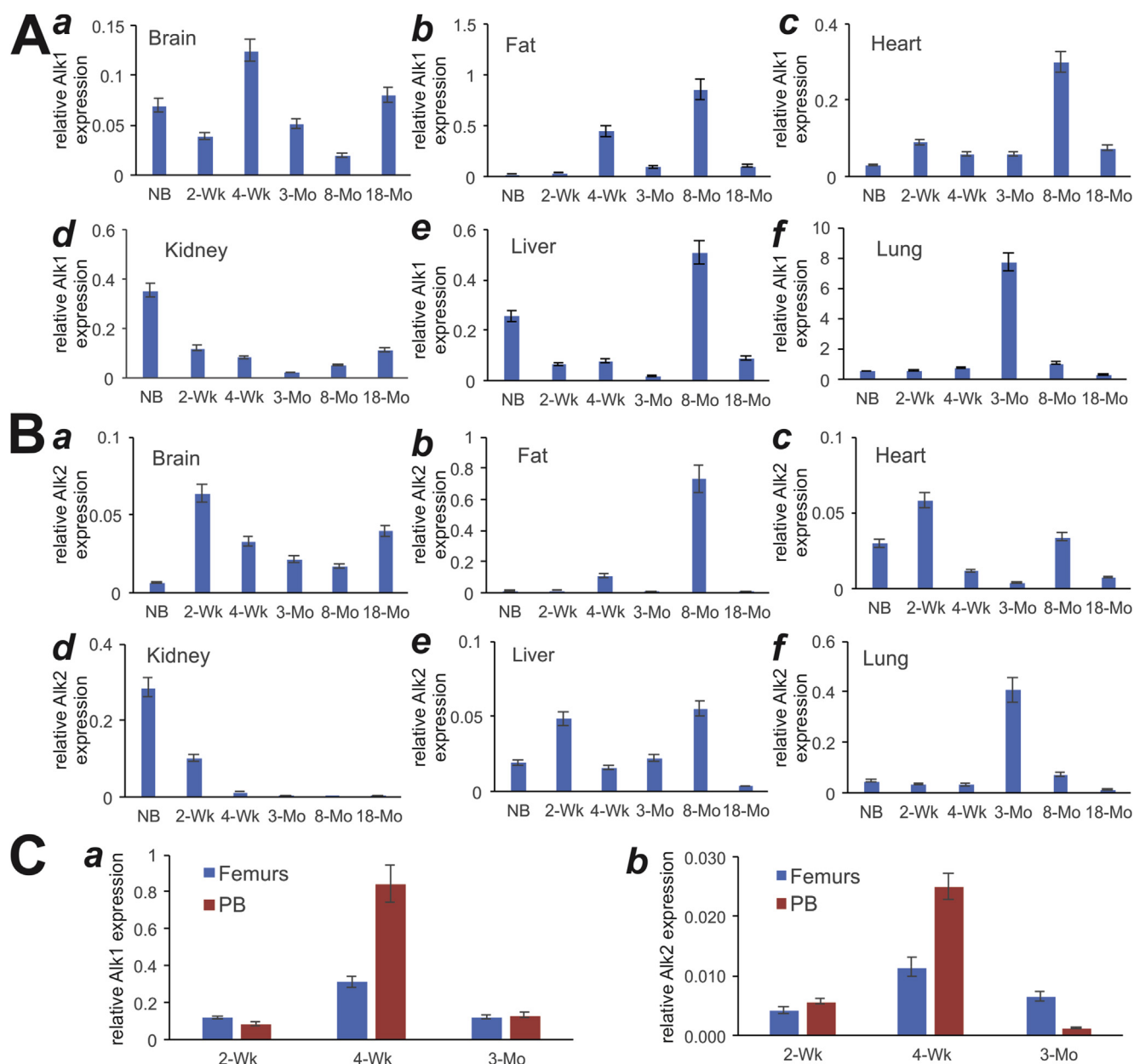


Figure 3 Expression of BMP9-associated receptors *Alk1* and *Alk2* in postnatal mouse tissues determined by TqPCR analysis. Total RNA was isolated from mouse brain, fat (inguinal region), heart, kidney, liver, lung, muscle, spleen, femur, and parietal bone (PB) at various ages: newborn (NB), 2-week, 4-week, 3-month, 8-month and 18-month old. The RT cDNA products were subjected to TqPCR analysis of *Alk1* and *Alk2* expression in these tissues.

a & b). However, *Alk1* was expressed at much higher levels than *Alk2* at the three age groups, suggesting *Alk1* may play a more important role in developing bones.

The negative regulators of BMP9 signaling, *Smad6*, *Smad7* and *noggin*, are widely expressed in mouse postnatal tissues

As the feedback inhibitors *Smad6* and *Smad7* (or so-called I-Smads) can serve as indirect indicators of the status of BMP signaling activities including BMP9, we further analyzed the expression patterns of *Smad6* and *Smad7* in the mouse tissues. In brain tissue, the expression of both *Smad6* and

Smad7 was shown highest in the 4-week group (Fig. 4A panel a and Fig. 4B panel a). On the other hand, both *Smad6* and *Smad7* peaked their expression in the 8-month age group in fat, heart, and liver tissues (Fig. 4A panels b, c, & e, and Fig. 4B panels b, c, & e). *Smad6* and *Smad7* also exhibited similar expression patterns in kidney samples with the highest expression at the 2-week age group (Fig. 4A panel d, and Fig. 4B panel d). However, the expression of *Smad6* and *Smad7* in the lungs seemingly increased with ages within the first 8 and 3 months after birth, respectively (Fig. 4A, panel f; and Fig. 4B panel f).

We further analyzed the expression of I-Smads in developing bone tissues and found that both *Smad6* and *Smad7* peaked their expression in femurs and parietal bone

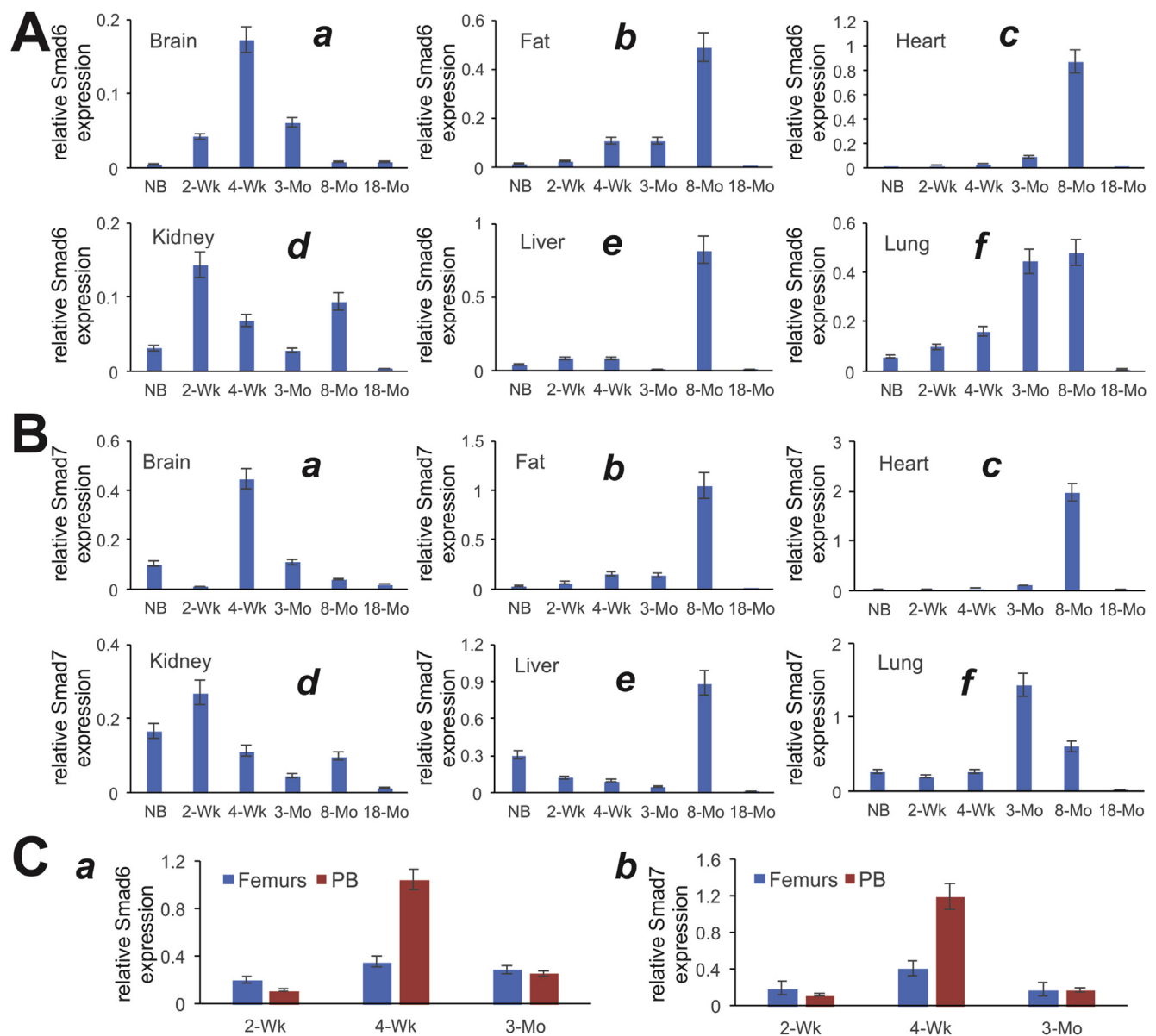


Figure 4 Expression of BMP9 signaling feedback inhibitors, *Smad6* and *Smad7*, in postnatal mouse tissues determined by TqPCR analysis. Total RNA was isolated from mouse brain, fat (inguinal region), heart, kidney, liver, lung, muscle, spleen, femur, and parietal bone (PB) at various ages: newborn (NB), 2-week, 4-week, 3-month, 8-month, and 18-month old. The RT cDNA products were subjected to TqPCR analysis of *Smad6* and *Smad7* expression in these tissues.

at the 4-week age group (Fig. 4C panels a & b), suggesting that BMP signaling, which is negatively regulated by *Smad6* and *Smad7*, may play an important role in developing bones.

Lastly, we analyzed the expression of the BMP signaling antagonist noggin. We found that noggin was expressed at low levels in the brain tissues (Fig. 5A panel a). In fat and heart tissues, noggin was shown to express at older age groups (e.g., 4-week to 8-month) (Fig. 5A, panels b & c). On the other hand, in kidney, liver and lung tissues noggin showed biphasic expression, expressed at high levels at birth and then dropped sharply with gradual increases with age (Fig. 5A, panels d, e, & f). However, in the developing bone tissues noggin expression increased with ages, from 2-week, 4-week to 3-month in femur and parietal bone

tissues (Fig. 5B, panel a & b). Taken together, these results indicate that negative regulators of BMP9 signaling, *Smad6*, *Smad7* and *noggin*, are widely expressed in mouse postnatal tissues.

BMP9 exhibits pleiotropic biological functions and yet is primarily expressed in postnatal liver and lungs

Increasing evidence indicates that BMP9 plays diverse arrays of biological functions in development and adult tissue homeostasis. However, little is known about the *in vivo* expression landscape of BMP9 and its signaling mediators. We analyzed the mouse ENCODE transcriptome dataset,

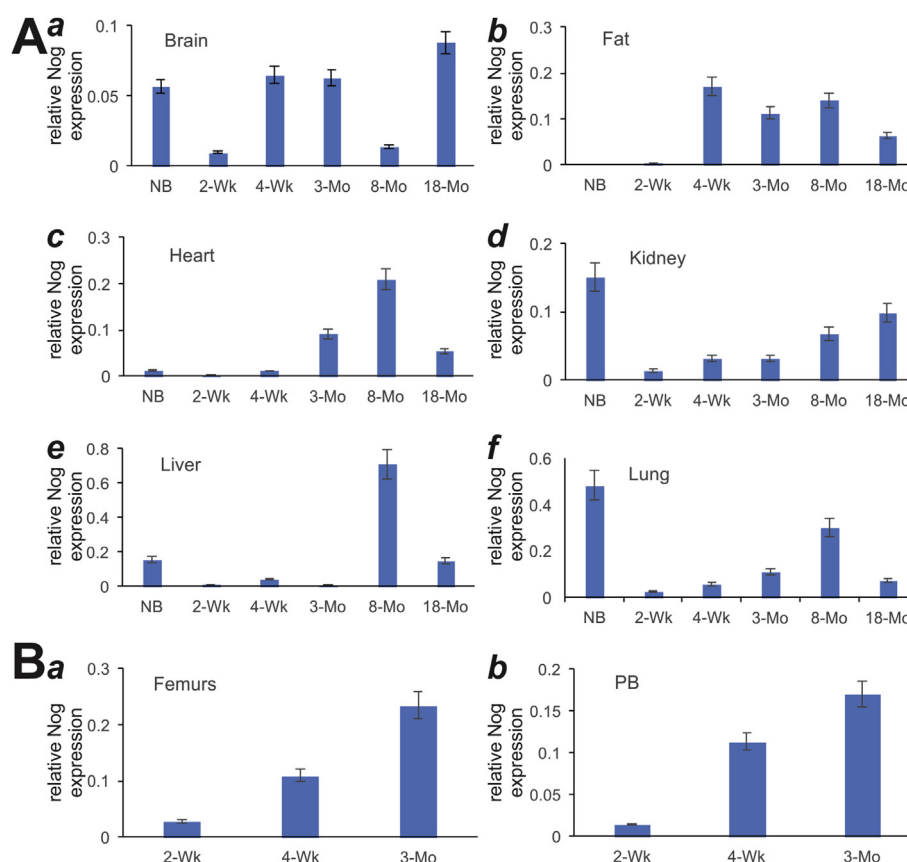


Figure 5 Expression of BMP9 signaling inhibitor, *Noggin*, in postnatal mouse tissues determined by TqPCR analysis. Total RNA was isolated from mouse brain, fat (inguinal region), heart, kidney, liver, lung, muscle, spleen, femur, and parietal bone (PB) at various ages: newborn (NB), 2-week, 4-week, 3-month, 8-month and 18-month old. The RT cDNA products were subjected to TqPCR analysis of *Noggin* expression in these tissues.

which contains 30 mouse samples of embryonic and adult tissues, and found that *Bmp9* was highly expressed in E14, E14.5, E18 liver and adult liver, although *Bmp9* expression was detectable in E11.5 brain, adult lungs and adult placenta. The ENCODE dataset further revealed that BMP9 receptor *Alk1* was highly expressed in adult lung tissue while *Alk2* was more broadly expressed, and that adult lung tissue had the highest expression for both I-Smads, followed by various GI tissues, although *Smad7* is more widely expressed than *Smad6*. Thus, the mouse ENCODE transcriptome data suggest that *Bmp9* expression may be relatively restricted to liver, and to a lesser extent, lungs, while the *Bmp9* signaling mediators, especially *Alk1*, *Smad6* and *Smad7*, exhibit high expression levels in adult lungs.

To overcome the ENCODE's limitations in scope and depth, we conducted a transcriptomic analysis of BMP9 signaling in major mouse organs including brain, fat, heart, kidney, liver, lungs, muscle, spleen, femur, and parietal bone at different ages ranging from newborn, 2-week, 4-week, 3-month, 8-month to 18-month old mice. Our results demonstrated that, although at relatively low levels at birth, *Bmp9* was highly expressed in the liver and lung tissues of young adult mice, but decreased in older mice. *Bmp9* expression was also detectable in 2-week spleen, brain and fat tissues and muscle samples. Interestingly, *Bmp9* was only expressed at low to modest levels in developing bones.

Furthermore, BMP9-specific BMP-R type I receptor *Alk1* was highly expressed in the adult lungs, while *Alk2* was expressed at relatively higher levels in fat and kidney tissues. The I-Smads *Smad6* and *Smad7* were widely expressed in mouse postnatal tissues, including developing bones. The naturally occurring antagonist of BMP signaling, *noggin*, was expressed highly in fat and heart tissues at older age groups, as well as in kidney, liver and lung tissues in a biphasic fashion. It seems that the expression patterns of *noggin* were independent of that of BMP9, *Alk1* and/or I-Smads in adult mice, which is consistent with our earlier findings about the refractory nature of BMP9 against *noggin*.²⁰

Emerging evidence indicates that BMP9 exerts diverse and pleiotropic biological functions during postnatal development and in maintaining adult tissue homeostasis. The postnatal expression landscape of BMP9 and its important signaling mediators should provide important insights into the potential functions of BMP9 signaling in major organs and/or tissues. We have recently conducted a comprehensive transcriptomic profiling of the MSCs stimulated by the 14 types of BMPs at the early stage, which allows us to identify the immediate early transcriptomic changes in MSCs.⁴⁸ The present study indicates that the circulating BMP9 produced in liver and lungs may account for its pleiotropic effects on multiple postnatal tissues/organs. Furthermore, the expression profiling analysis

strongly suggests that BMP9 may play important roles in regulating hepatic metabolism and tissue homeostasis and pulmonary endothelial cells of the lungs,^{10,38,39} which warrants thorough future investigations.

Conflict of interest

The authors declare no competing interest.

Acknowledgments

The reported work was supported in part by research grants from the National Institutes of Health (CA226303 to TCH), the U.S. Department of Defense (OR130096 to JMW), the Scoliosis Research Society (TCH and MJL). This project was also supported in part by The University of Chicago Cancer Center Support Grant (P30CA014599) and the National Center for Advancing Translational Sciences of the National Institutes of Health through Grant Number UL1 TR000430. TCH was also supported by the Mabel Green Myers Research Endowment Fund and The University of Chicago Orthopaedics Alumni Fund. Funding sources were not involved in the study design; in the collection, analysis and interpretation of data; in the writing of the report; and in the decision to submit the paper for publication.

References

- Hogan BL. Bone morphogenetic proteins: multifunctional regulators of vertebrate development. *Genes Dev.* 1996;10(13):1580–1594.
- Reddi AH. Bone morphogenetic proteins: an unconventional approach to isolation of first mammalian morphogens. *Cytokine Growth Factor Rev.* 1997;8(1):11–20.
- Attisano L, Wrana JL. Signal transduction by the TGF-beta superfamily. *Science.* 2002;296(5573):1646–1647.
- Wang RN, Green J, Wang Z, et al. Bone Morphogenetic Protein (BMP) signaling in development and human diseases. *Gene Dis.* 2014;1(1):87–105.
- Deng ZL, Sharff KA, Tang N, et al. Regulation of osteogenic differentiation during skeletal development. *Front Biosci.* 2008;13:2001–2021.
- Kim JH, Liu X, Wang J, et al. Wnt signaling in bone formation and its therapeutic potential for bone diseases. *Ther Adv Musculoskelet Dis.* 2013;5(1):13–31.
- Denduluri SK, Idowu O, Wang Z, et al. Insulin-like growth factor (IGF) signaling in tumorigenesis and the development of cancer drug resistance. *Gene Dis.* 2015;2(1):13–25.
- Teven CM, Farina EM, Rivas J, Reid RR. Fibroblast growth factor (FGF) signaling in development and skeletal diseases. *Gene Dis.* 2014;1(2):199–213.
- Lamplot JD, Qin J, Nan G, et al. BMP9 signaling in stem cell differentiation and osteogenesis. *Am J Stem Cells.* 2013;2(1):1–21.
- Luther G, Wagner ER, Zhu G, et al. BMP-9 induced osteogenic differentiation of mesenchymal stem cells: molecular mechanism and therapeutic potential. *Curr Gene Ther.* 2011;11(3):229–240.
- Zhao GQ. Consequences of knocking out BMP signaling in the mouse. *Genesis.* 2003;35(1):43–56.
- Shi Y, Massague J. Mechanisms of TGF-beta signaling from cell membrane to the nucleus. *Cell.* 2003;113(6):685–700.
- Luu HH, Song WX, Luo X, et al. Distinct roles of bone morphogenetic proteins in osteogenic differentiation of mesenchymal stem cells. *J Orthop Res.* 2007;25(5):665–677.
- Urist MR. Bone: formation by autoinduction. *Science.* 1965;150(3698):893–899.
- Wozney JM, Rosen V, Celeste AJ, et al. Novel regulators of bone formation: molecular clones and activities. *Science.* 1988;242(4885):1528–1534.
- Cheng H, Jiang W, Phillips FM, et al. Osteogenic activity of the fourteen types of human bone morphogenetic proteins (BMPs). *J Bone Joint Surg Am.* 2003;85(8):1544–1552.
- Kang Q, Sun MH, Cheng H, et al. Characterization of the distinct orthotopic bone-forming activity of 14 BMPs using recombinant adenovirus-mediated gene delivery. *Gene Ther.* 2004;11(17):1312–1320.
- Huang X, Wang F, Zhao C, et al. Dentinogenesis and tooth-alveolar bone complex defects in BMP9/GDF2 knockout mice. *Stem Cells Dev.* 2019;28(10):683–694.
- Zhang F, Song J, Zhang H, et al. Wnt and BMP signaling cross-talk in regulating dental stem cells: implications in dental tissue engineering. *Gene Dis.* 2016;3(4):263–276.
- Wang Y, Hong S, Li M, et al. Noggin resistance contributes to the potent osteogenic capability of BMP9 in mesenchymal stem cells. *J Orthop Res.* 2013;31(11):1796–1803.
- Luo J, Tang M, Huang J, et al. TGFbeta/BMP type I receptors ALK1 and ALK2 are essential for BMP9-induced osteogenic signaling in mesenchymal stem cells. *J Biol Chem.* 2010;285(38):29588–29598.
- Peng Y, Kang Q, Cheng H, et al. Transcriptional characterization of bone morphogenetic proteins (BMPs)-mediated osteogenic signaling. *J Cell Biochem.* 2003;90(6):1149–1165.
- Peng Y, Kang Q, Luo Q, et al. Inhibitor of DNA binding/differentiation helix-loop-helix proteins mediate bone morphogenetic protein-induced osteoblast differentiation of mesenchymal stem cells. *J Biol Chem.* 2004;279(31):32941–32949.
- Luo Q, Kang Q, Si W, et al. Connective tissue growth factor (CTGF) is regulated by Wnt and bone morphogenetic proteins signaling in osteoblast differentiation of mesenchymal stem cells. *J Biol Chem.* 2004;279(53):55958–55968.
- Sharff KA, Song WX, Luo X, et al. Hey1 basic helix-loop-helix protein plays an important role in mediating BMP9-induced osteogenic differentiation of mesenchymal progenitor cells. *J Biol Chem.* 2009;284(1):649–659.
- Huang E, Zhu G, Jiang W, et al. Growth hormone synergizes with BMP9 in osteogenic differentiation by activating the JAK/STAT/IGF1 pathway in murine multilineage cells. *J Bone Miner Res.* 2012;27(7):1566–1575.
- Zhang J, Weng Y, Liu X, et al. Endoplasmic reticulum (ER) stress inducible factor cysteine-rich with EGF-like domains 2 (Crelid2) is an important mediator of BMP9-regulated osteogenic differentiation of mesenchymal stem cells. *PLoS One.* 2013;8(9), e73086.
- Hu N, Jiang D, Huang E, et al. BMP9-regulated angiogenic signaling plays an important role in the osteogenic differentiation of mesenchymal progenitor cells. *J Cell Sci.* 2013;126(Pt 2):532–541.
- Tang N, Song WX, Luo J, et al. BMP-9-induced osteogenic differentiation of mesenchymal progenitors requires functional canonical Wnt/beta-catenin signalling. *J Cell Mol Med.* 2009;13(8B):2448–2464.
- Zhang W, Deng ZL, Chen L, et al. Retinoic acids potentiate BMP9-induced osteogenic differentiation of mesenchymal progenitor cells. *PLoS One.* 2010;5(7), e11917.
- Chen L, Jiang W, Huang J, et al. Insulin-like growth factor 2 (IGF-2) potentiates BMP-9-induced osteogenic differentiation and bone formation. *J Bone Miner Res.* 2010;25(11):2447–2459.

32. Liu X, Qin J, Luo Q, et al. Cross-talk between EGF and BMP9 signalling pathways regulates the osteogenic differentiation of mesenchymal stem cells. *J Cell Mol Med*. 2013;17(9):1160–1172.
33. Zhang H, Wang J, Deng F, et al. Canonical Wnt signaling acts synergistically on BMP9-induced osteo/odontoblastic differentiation of stem cells of dental apical papilla (SCAPs). *Biomaterials*. 2015;39:145–154.
34. Li R, Zhang W, Cui J, et al. Targeting BMP9-promoted human osteosarcoma growth by inactivation of notch signaling. *Curr Cancer Drug Targets*. 2014;14(3):274–285.
35. Zhang H, Li L, Dong Q, et al. Activation of PKA/CREB signaling is involved in BMP9-induced osteogenic differentiation of mesenchymal stem cells. *Cell Physiol Biochem*. 2015;37(2):548–562.
36. Liao J, Wei Q, Zou Y, et al. Notch signaling augments BMP9-induced bone formation by promoting the osteogenesis-angiogenesis coupling process in mesenchymal stem cells (MSCs). *Cell Physiol Biochem*. 2017;41(5):1905–1923.
37. Liao J, Yu X, Hu X, et al. lncRNA H19 mediates BMP9-induced osteogenic differentiation of mesenchymal stem cells (MSCs) through Notch signaling. *Oncotarget*. 2017;8(32):53581–53601.
38. Mostafa S, Pakvasa M, Coalson E, et al. The Wonders of BMP9: from mesenchymal stem cell differentiation, angiogenesis, neurogenesis, tumorigenesis, and metabolism to regenerative medicine. *Gene Dis*. 2019;6(3):201–223.
39. Toshner M. BMP9 morphs into a potential player in portopulmonary hypertension. *Am J Respir Crit Care Med*. 2019;199(7):819–821.
40. Song JJ, Celeste AJ, Kong FM, Jirtle RL, Rosen V, Thies RS. Bone morphogenetic protein-9 binds to liver cells and stimulates proliferation. *Endocrinology*. 1995;136(10):4293–4297.
41. Yue F, Cheng Y, Breschi A, et al. A comparative encyclopedia of DNA elements in the mouse genome. *Nature*. 2014;515(7527):355–364.
42. Untergasser A, Cutcutache I, Koressaar T, et al. Primer3-new capabilities and interfaces. *Nucleic Acids Res*. 2012;40(15):e115.
43. Zhang Q, Wang J, Deng F, et al. TqPCR: a touchdown qPCR assay with significantly improved detection sensitivity and amplification efficiency of SYBR green qPCR. *PLoS One*. 2015;10(7), e0132666.
44. Zhao C, Zeng Z, Qazvini NT, et al. Thermoresponsive citrate-based graphene oxide scaffold enhances bone regeneration from BMP9-stimulated adipose-derived mesenchymal stem cells. *ACS Biomater Sci Eng*. 2018;4(8):2943–2955.
45. Fan J, Wei Q, Liao J, et al. Noncanonical Wnt signaling plays an important role in modulating canonical Wnt-regulated stemness, proliferation and terminal differentiation of hepatic progenitors. *Oncotarget*. 2017;8(16):27105–27119.
46. Hu X, Li L, Yu X, et al. CRISPR/Cas9-mediated reversibly immortalized mouse bone marrow stromal stem cells (BMSCs) retain multipotent features of mesenchymal stem cells (MSCs). *Oncotarget*. 2017;8(67):111847–111865.
47. Zhao C, Jiang W, Zhou N, et al. Sox9 augments BMP2-induced chondrogenic differentiation by downregulating Smad7 in mesenchymal stem cells (MSCs). *Gene Dis*. 2017;4(4):229–239.
48. Zhang L, Luo Q, Shu Y, et al. Transcriptomic landscape regulated by the 14 types of bone morphogenetic proteins (BMPs) in lineage commitment and differentiation of mesenchymal stem cells (MSCs). *Gene Dis*. 2019.