BIOGRAPHICAL SKETCH

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NAME: Mishina, Yuji

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POSITION TITLE: Professor

EDUCATION/TRAINING

INSTITUTION AND LOCATION	DEGREE *(if applicable)*	Completion Date MM/YYYY	FIELD OF STUDY
University of Tokyo, Tokyo	BS	03/1981	Biology
University of Tokyo, Tokyo	MS	03/1983	Molecular Biology
University of Tokyo, Tokyo	PHD	03/1986	Molecular Biology

A. Personal Statement

My laboratory has been extensively involved in studies utilizing genetically altered mice to investigate the mechanisms of tissue formation and organogenesis, in particular, bone and cartilage. Cell fate decisions of embryonic and adult stem cells are intimately linked to these processes and are also investigated. We have developed tissue-specific gene knockouts as well as overexpression mouse systems that can also control gene alteration in a time dependent manner. While investigating interactions of Bone Morphogenetic Protein (BMP) signaling with other growth factors such as fibroblast growth factor (FGF), Wnt and Hedgehog, we have determined that the extent and nature of interplay is highly context dependent. We have also found that BMP signaling in osteoblasts regulates bone formation through multiple processes, and BMP signaling functions in neural crest stem cells playing a critical role in directing cell fate toward the chondrocyte lineage. I have trained more than 50 predoctoral and postdoctoral fellows, and most are engaged in productive academic careers. For the past 10 years, I have mentored 18 postdocs and 16 of them continue research in academia, one has become a clinician oncologist and one has become an educator in a public school system. I have mentored and am mentoring three graduate students (I served one of them as a primary co-mentor) and all three have received F30 awards. Another graduate student I am co-mentoring recently received F30. I served as a mentor in the DSPP-K12 program a few years ago to enjoy mentoring junior faculties (2015-2017). One of my trainees, Dr. Yoshihiro Komatsu received K99/R00 award in 2010 and he was recently promoted to Associate Professor with tenure at UTHealth Houston McGovern Medical School. I am serving as a co-mentor of following individuals for their K99/R00; Qian Cong (Harvard School of Dental Medicine) and Shawon Debnath (Weill Cornell Medicine).

Our group has more than 20 years of experience to investigate unique functions of each BMP receptor signaling in different tissues including the skeletal system. Most of the knockout and transgenic mice we are using are produced by me and are used worldwide. We have developed a transgenic mouse line that conditionally enhances BMP-Smad signaling and demonstrated at the first time that in combination with trauma, this mouse line develops heterotopic ossification (HO). This mouse line now became a world standard, and several pharmaceutical companies are using this line to screen small molecule inhibitors that can prevent HO. We have developed another transgenic mouse line that conditionally enhance BMP-Smad signaling and demonstrated that this mouse line develops craniofacial abnormalities including premature fusion of cranial sutures, hypomorphic cranial cartilage formation and massive formation of ectopic cartilage in the facial area.

In this DSPP-K12 program, I will play a pivotal role to organize and implement the planned education and trainings. I have over two decades of experiences to train multiple levels of trainees (20 PhD students as primary/secondary thesis advisor, 12 dental specialty master students as primary/secondary thesis advisor, 55 undergraduate students as a primary mentor, 28 postdoctoral fellows as a primary mentor, 12 visiting scholars and 12 junior faculty members). In addition to these mentoring activities, I took *Strategies and Tactics for Recruiting to Improve Diversity and Excellence* (STRIDE) training in 2019 to learn about DEI and potential bias. I have attended RCRS activity in every other year and served as an instructor in 2018 for image

processing and in 2020 and 2022 for academic collaboration. Together with my over two decades of experience as a developmental mouse geneticist in craniofacial/skeletal biology will significantly empower the DSPP-K12 program training grant and thus I hope that my contribution will lead to valuable outcomes.

1. Yang, J., Kitami, M., Pan, H., Toda Nakamura, M., Zhang, H., Liu, F., Zhu, L., Komatsu, Y., and **Mishina, Y.**: Augmented BMP signaling commits cranial neural crest cells to a chondrogenic fate via suppressing autophagic β-catenin degradation. *Science Signaling*. 2021 14(665):eaaz9368. PubMed PMID: 32622875; PubMed Central PMCID: PMC7936468.
2. Swanson, W.B., Omi, M., Zhang, Z., Nam, H.K., Wang, G., Ma, P.X., Hatch N.E., and **Mishina, Y**: Macropore design of tissue engineering scaffolds regulates mesenchymal stem cell differentiation fate. Biomaterials. 272:120769, 2021. (Published online March 24, 2021.) PubMed PMID: 33798961; PubMed Central PMCID: PMC1686925.
3. Hayano S, Komatsu Y, Pan H, **Mishina Y**. Augmented BMP signaling in the neural crest inhibits nasal cartilage morphogenesis by inducing p53-mediated apoptosis. *Development*. 2015 Apr 1;142(7):1357-67. PubMed PMID: 25742798; PubMed Central PMCID: PMC4378250.
4. Yu PB, Deng DY, Lai CS, Hong CC, Cuny GD, Bouxsein ML, Hong DW, McManus PM, Katagiri T, Sachidanandan C, Kamiya N, Fukuda T, **Mishina Y**, Peterson RT, Bloch KD. BMP type I receptor inhibition reduces heterotopic [corrected] ossification. *Nat Med*. 2008 Dec;14(12):1363-9. PubMed PMID: 19029982; PubMed Central PMCID: PMC2846458.

B. Positions, Scientific Appointments and Honors

Positions and Employment

2022-	Associated Director of the OHS-PhD program
2022-	William R Mann Professor of Dentistry, University of Michigan, Ann Arbor, MI
2014-	Professor, University of Michigan, Ann Arbor, MI
2008-2014	Associate Professor, University of Michigan, Ann Arbor, MI
1998-2008	Section Head, NIEHS, RTP, NC
1992-1998	Postdoctoral Fellow, The University of Texas, MD Anderson Cancer Center, Houston, TX
1986-1992	Research Associate, Institute for Biological Research, Yokohama City University, Yokohama

Scientific Appointments, Professional Memberships, and Other Experience

2019-	Member, American Society for Bone Mineral Research
2015-2020	Visiting Professor (honorary), Jilin University School of Stomatology, China,
2015-2017	High End Foreign Experts Recruiting Program, Jilin University School of Stomatology, China
2014-	Associate Editor, genesis
2014-	Member, American Association of Dental Research
2011-2015	Editorial Board, J. of Bone and Mineral Research
2011-	Editorial Board, genesis
2010-	Editorial Board, Frontiers in Craniofacial Biology
2009-2014	Editorial Board, The Open Bio Markers
2009-2014	Editorial Board, J. Dental Research
2007-2012	Editorial Board, Sexual Development
1999-	Member, Society for Developmental Biology

Honors

2022	Distinguished Mentor Award, University of Michigan, School of Dentistry
2018	M-Cubed 3.0 Seed Funding Award, University of Michigan
2016	M-Cubed 2.0 Seed Funding Award, University of Michigan
2013	M-Cubed Seed Funding Award, University of Michigan
2009	Office of Vice President for Research, Award for Small Scale and Preliminary Projects, University of Michigan

C. Contribution to Science

1. Dual functions of BMP signaling in osteoblasts for skeletal homeostasis. To understand BMP signaling during skeletogenesis and bone remodeling, we mutated Bmpr1a in an osteoblast-specific manner using a tamoxifen-inducible-Cre system (conditional knockout, cKO). In results that were totally opposite from what was expected, mineralization was increased in cKO bones. These results demonstrate that BMP signaling through BMPRIA negatively regulates bone mass. In the cKO bones, we found osteoclastogenesis is severely reduced, which can explain the increase of bone mass. We also found that canonical Wnt signaling in cKO mice was upregulated due to the reduction of Sclerostin and Dkk1, Wnt inhibitors. Thus, we conclude that BMP signaling positively regulates Sost expression to negatively regulate canonical Wnt signaling to balance bone formation and bone resorption. Our results from in vivo studies revealed that BMP functions on osteoblasts are far more complicated than expected, and thus prompted further molecular mechanistic studies for better application of BMP2 and BMP7, which are already used in clinics to assist fracture healing.
    a. Omi, M., Kaartinen, K., and **Mishina, Y**.: Activin A receptor type 1-mediated BMP signaling regulates RANKL-induced osteoclastogenesis via canonical SMAD-signaling pathway. J. Biol. Chem. 294:17818-17836, 2019. PubMed PMID: 31619522; PubMed Central PMCID: PMC6879329.
    b. Zhang, H., Zhang, Y., Terajima, M., Romanowicz, G., Yangjia Liu, Y., Omi, M., Bigelow, E., M. Joiner, D.M., Waldorff, E.I., Zhu, P., Raghavan, M., Lynch, M., Kamiya, N., Zhang, R., Jepsen, K.J., Goldstein, S., Morris, M.D., Yamauchi, M., H. Kohn, D.H., and **Mishina, Y**.: Loss of BMP signaling mediated by BMPR1A in osteoblasts leads to differential bone phenotypes depending on anatomical positions of the bones in mice. Bone. 2020 137, 115402. PubMed PMID: 32360900; PubMed Central PMCID: PMC1593181.
    c. Hsieh, H.H.S., Agarwal, S., Cholok, D.J., Chung, M.T, Ranganathan, K., Habbouche, J., Li, J., Butts, J., Reimer, J., Kaura, A., Drake, J., Breuler, C., Priest, C.R., Nguyen, J., Brownley, R.C., Ucer S., Niknafs, Y.S, Li, S., Inagaki, M., Scott, G., Krebsbach, P.H., Longaker, M.T., Westover, K., Gray, N., Ninomiya-Tsuji, J., Davis, T., **Mishina, Y**., and Levi, B. A Novel Strategy for Wound Healing through Gene in-activation and re-activation. 2019 Stem Cells. 37, 766-778. PubMed PMID: 30786091. PubMed Central PMCID: PMC6542699.
    d. Kamiya N, Kobayashi T, Mochida Y, Yu PB, Yamauchi M, Kronenberg HM, **Mishina Y**. Wnt inhibitors Dkk1 and Sost are downstream targets of BMP signaling through the type IA receptor (BMPRIA) in osteoblasts. J Bone Miner Res. 2010 Feb;25(2):200-10. PubMed PMID: 19874086; PubMed Central PMCID: PMC3153381.

2. Unexpected function of BMP on cranial neural crest differentiation. We mutated Bmpr1a in a neural crest cell-specific manner using P0-Cre mice. In combination with the Cre/loxP system, we established a transgenic line that conditionally overexpresses the constitutively-active form of Bmpr1a. To address the impact of enhanced BMP signaling in neural crest cells, we bred these mouse lines with P0-Cre mice. When BMPRIA signaling is enhanced in a neural crest-specific manner, the resulted mice developed craniosynostosis due to the premature suture fusion. Causative genes for human craniosynostosis are identified for only 20% of cases, and thus BMPRIA would be a good candidate for which mutations cause some of the unidentified cases.
    a. Yang J, Kitami M, Pan H, Toda Nakamura M, Zhang H, Liu F, Zhu L, Komatsu Y, and **Mishina, Y**: Augmented BMP signaling commits cranial neural crest cells to a chondrogenic fate via suppressing autophagic β-catenin degradation. Science Signaling. 2021 14(665):eaaz9368. PubMed PMID: 32622875; PubMed Central PMCID: PMC7936468.
    b. Hayano S, Komatsu Y, Pan H, **Mishina Y**. Augmented BMP signaling in the neural crest inhibits nasal cartilage morphogenesis by inducing p53-mediated apoptosis. Development. 2015 Apr 1;142(7):1357-67. PubMed PMID: 25742798; PubMed Central PMCID: PMC4378250.
    c. **Komatsu Y**, Yu PB, Kamiya N, Pan H, Fukuda T, Scott GJ, Ray MK, Yamamura K, **Mishina Y**. Augmentation of Smad-dependent BMP signaling in neural crest cells causes craniosynostosis in mice. J Bone Miner Res. 2013 Jun;28(6):1422-33. PubMed PMID: 23281127; PubMed Central PMCID: PMC3638058.
    d. Yumoto K, Thomas PS, Lane J, Matsuzaki K, Inagaki M, Ninomiya-Tsuji J, Scott GJ, Ray MK, Ishii M, Maxson R, **Mishina Y**, Kaartinen V. TGF-β-activated kinase 1 (Tak1) mediates agonist-induced Smad activation and linker region phosphorylation in embryonic craniofacial neural crest-derived cells. J Biol Chem. 2013 May 10;288(19):13467-80. PubMed PMID: 23546880; PubMed Central PMCID: PMC3650384.

3. Functions of BMP signaling that may lead to ciliopathy. Malfunctions in cilial functions and/or cilia formation may cause pathological conditions that are now categorized as ciliopathy. We previously found that loss of BMP signaling through ACVR1 leads to a right isomerism (both sides of body develop with a right-side identity to cause lethality). We uncovered that ACVR1 signaling negatively regulates cell proliferation at the node to create permissive condition for cilial formation, which is critical to establish the left-right asymmetry. This is the first evidence that BMP signaling is directly involved in ciliogenesis. We also developed a mouse model to overexpress ACVR1 signaling and found that neural crest-specific activation of ACVR1 leads to ectopic cartilage formation associated with increased cilial formation. These findings prompted us to hypothesis that levels of BMP signaling through ACVR1 is critical for normal ciliogenesis and thus misregulation of the ACVR1 may lead to ciliopathy. To further elucidate ciliopathy through a different approach, we generated a model mouse for Elis van-Creveld (EvC) syndrome, a rare recessive congenital disease characterized by chondrodysplastic dwarfism. EVC2, a product from the causative gene of EvC syndrome, is localized in cilia and we found that growth factor signaling such as hedgehog, Wnt, FGF and BMP pathways is altered in cartilages in developing long bones. Analyses of this newly generated mouse model should shed light on the cause-effect relations between growth factor signaling and cilial functions and thus provide deeper understanding for a better treating ciliopathies.
    a. Zhang, H.,* Louie, K.A., Kulkarni, A., Zapien-Guerra, K., Yang, J., and **Mishina, Y.*:** The posterior part influences the anterior part of the mouse cranial base development. JBMPR Plus. 6:e10589, 2021. (Accepted, Nov. 29, 2021. Published online Dec. 06, 2021) *, co-corresponding. PubMed PMID: 35229066; PubMed Central PMCID: PMC8861986
    b. Kulkarni A, Louie KA, Mochida Y, Cevidanes L, **Mishina Y**, and Zhang H: A ciliary protein EVC2/LIMBIN plays a critical role in the skull base for mid-facial development. Frontier of Phys. (Accepted on Oct. 1, 2018, published online Oct. 25, 2018). PubMed PMID: 30410447; PubMed Central PMCID: PMC6210651.
    c. Zhang H, Kamiya N, Tsuji T, Takeda H, Scott G, Rajderkar S, Ray MK, Mochida Y, Allen B, Lefebvre V, Hung IH, Ornitz DM, Kunieda T, **Mishina Y**. Elevated Fibroblast Growth Factor Signaling Is Critical for the Pathogenesis of the Dwarfism in Evc2/Limbin Mutant Mice. PLoS Genet. 2016 Dec 27;12(12):e1006510. PubMed PMID: 28027321; PubMed Central PMCID: PMC5189957.
    d. Zhang H, Takeda H, Tsuji T, Kamiya N, Kunieda T, Mochida Y, **Mishina Y**. Loss of Function of Evc2 in Dental Mesenchyme Leads to Hypomorphic Enamel. J Dent Res. 2017 Apr;96(4):421-429. PubMed PMID: 28081373; PubMed Central PMCID: PMC5384488.

4. Elucidating the pathways and treatments behind heterotopic ossification. Having developed the mouse that is considered the gold standard to study heterotopic ossification, I have actively investigated the pathway, diagnosis and potential treatment strategies for this disabling process. Together with Dr. Benjamin Levi, we have made great strides in understanding the progenitor cells, pathways, diagnostic strategies and possible treatments for genetic heterotopic ossification (fibrodysplasia ossificans progressive) and traumatic heterotopic ossification.
    a. Agarwal S, Loder S, Cholok D, Li J, Bian G, Li S, Carson W, Delano M, Standiford, TJ, Kunkel S, **Mishina, Y**, Ward, P, and Levi, B: Disruption of neutrophil extracellular traps (NETs) links mechanical strain to post-injury inflammation and wound pathology. 2019 Frontiers in Immunology. 10:2148. PubMed PMID: 31708911; PubMed Central PMCID: PMC6821718.
    b. Agarwal S, Loder SJ, Sorkin M, Li S, Shrestha S, Zhao B, **Mishina Y**, James AW, Levi B. Analysis of Bone-Cartilage-Stromal Progenitor Populations in Trauma Induced and Genetic Models of Heterotopic Ossification. Stem Cells. 2016 Jun;34(6):1692-701. PubMed PMID: 27068890; PubMed Central PMCID: PMC4892971.
    c. Agarwal S, Loder S, Brownley C, Cholok D, Mangiavini L, Li J, Breuler C, Sung HH, Li S, Ranganathan K, Peterson J, Tompkins R, Herndon D, Xiao W, Jumlongras D, Olsen BR, Davis TA, **Mishina Y**, Schipani E, Levi B. Inhibition of Hif1α prevents both trauma-induced and genetic heterotopic ossification. Proc Natl Acad Sci U S A. 2016 Jan 19;113(3):E338-47. PubMed PMID: 26721400; PubMed Central PMCID: PMC4725488.
    d. Sorkin M, Huber AM, Hwang C, Carson IV WF, Menon R, Li J, Vasquez, K, Pagani C, Patel N, Li S, Visser ND, Niknafs Y, Loder S, Scola M, Nycz D, Gallagher K, McCauley LK, Xu J, James AW, Agarwal S, Kunkel S, **Mishina Y**, and Levi B: Regulation of heterotopic ossification by monocytes in a mouse

model of aberrant wound healing. Nature Communication. 11:722, 2020. PubMed PMID: 32024825; PubMed Central PMCID: PMC7002453.