in vitro culture. In general, cells that express CD44, CD90,

Interestingly, administration time point has been recently shown to significantly influence the effects of BMPs on the osteogenic differentiation of ASCs. When vitamin D_3 is constantly treated, BMP-2 is more efficacious to induce the osteogenesis of ASCs when treated for the last 7 days (8?4 days) than the first 7 days (1? days). [42] It seems plausible that specific compositions in these media determine the differentiation direction of ASCs, and then BMPs can significantly both enhance and accelerate the process.

The possibility of coadministering BMP-2 with other cytokines to achieve synergistic effects has also been investigated. Retinoic acid (RA) can simultaneously promote the osteogenic differentiation and inhibit the adipogenic differentiation of murine ASCs. Coapplication of RA and BMP-2 is reported to synergistically promote the osteogenic differentiation of murine ASCs *in vitro*.[43] In addition, combined treatment with BMP-2 and vitamin D_3 can synergistically induce the osteogenic differentiation of human ASCs *in vitro*.[42]

Traditionally, *in vitro* pretreatment with osteogenic medium is necessary to commit the osteogenic differentiation of ASCs before transplantation *in vivo*. However, this method is not ideal because the long-term culture *in vitro* will increase the risks of contamination and the possibility of change in biological behavior of cells, and significantly compromise the application potential to some urgent clinical cases.[44] Therefore, *in vivo* osteogenic induction in ASCs by BMPs has recently become an important research focus for ASCs-based bone tissue engineering. BMPs can be administrated by a simple subcutaneous injection for 1? days [44] or preintegration into scaffolds.[45] The latter has the advantage in aspects of cost-effectiveness and clinician-friendliness.

However, to exert their optimal osteoinductive efficacy, BMPs need to be gradually delivered to the target site, at a low level and in a sustained manner, instead of in a single high-dose burst.[1] Therefore, many efforts have been performed to develop slow-delivery systems to significantly enhance the efficiency of BMPs in promoting the osteogenic differentiation of ASCs *in vitro* and *in vivo*. ASCs and the BMP-slow-delivery scaffolds synergistically enhance the osteogenic events. Composite scaffolds that comprise of organic and inorganic phases are especially promising for inducing osteogenesis of ASCs. For example, a HA/b-tricalcium phosphate (b-TCP) scaffold that can facilitate the sustained release of BMP-2 over a 20-day period has been shown to significantly augment the osteogenic differentiation of ASCs *in vitro*.[46] In addition, many other scaffolds with similar properties have shown promise in this type of application, for example, a poly (DL-lactic-coglycolic acid) (PLGA) scaffold with a fibrin/hyaluronic acid coating [47] and a gelatin/b-TCP scaffold.[48] These slow-release systems can stimulate osteogenic differentiation, extracellular matrix deposition, maturation, and mineralization of ASCs.[49] Further, besides the slowly released BMP-2, the scaffold biomaterial itself may also play a role in the osteogenic differentiation of ASCs *in vitro*.[48] These benefits can be attributed to the interaction between BMP-2 and b-TCP: BMP-2 can increase the dissolution of b-TCP, while b-TCP can resorb BMP-2 from media and provide Ca^{2+} and PO_4^{3-} that are needed for bone mineralization.[45]

Consistent with the findings of *in vitro* studies, a controlled-released system is thought to maximize the promoting effect of BMPs on the *in vivo* osteogenesis of ASCs. For example, a PLGA/HA composite scaffold is capable of releasing BMP-2 over a 4-week period *in vitro* and thereby stimulates bone regeneration following transplantation of undifferentiated human ASCs *in vivo*.[49] This method avoids an *in vitro* culture period, and thus maximally favors the application potential of ASCs in clinic. The contribution of the transplanted human ASCs to newly formed bone is corroborated by the presence of human nuclear antigen-positive cells and the expression of specific human osteogenic proteins in the area of new bone formation in nude mice models.[2,44,49,50]

Gene technology

Over the past decade, BMPs and their signaling have also been introduced using gene technologies to induce the osteogenic differentiation of ASCs. These gene technologies include gene transfection of BMPs and the key BMP signaling components, and gene knockdown of the BMP antagonists (Fig. 1). Human ASCs transfected with *BMP-2* have been shown to significantly promote bone formation in many animal models, including ectopic bone induction in mice,[51?3] critical-size bone defects of rats,[54,55] and spine fusion in rats.[56] Further, cotransfection of *RUNX2* or *VEGF* with *BMP-2* is shown to enhance bone regeneration and accelerate the healing of segmental defects more effectively than *BMP-2* alone *in vivo*.[57,58] In addition to *BMP-2*, significant bone formation can also be achieved by transfection of ASCs with *BMP-4*,[32,33] *BMP-6*,[5] or *BMP-7*.[59,60]

On the other hand, the gene knockdown of *noggin*, one of the BMP antagonists, significantly upregulates BMP signaling and enhances BMP-2-induced osteogenic differentiation of ASCs *in vitro* and *in vivo*.[2,61] Interestingly, *noggin* knockdown also increases angiogenesis, which is essential for bone formation.[61] Moreover, simultaneous overexpression of *BMP-2* and repression of *noggin* synergistically enhance the osteogenesis of ASCs.[62]

The induction of endogenous BMPs

Many active agents can upregulate endogenous BMPs (Fig. 2), which at least partially accounts for their promoting effect on the osteogenesis of ASCs. The expression of endogenous BMPs, such as BMP-2, BMP-4, and BMP-6, can be detected in osteogenic medium.[3,37,38] The expression level of endogenous BMPs is also adopted as an important parameter to evaluate the potency of an osteogenic medium.[3,38] The types of endogenous BMPs are also dependent on the types of active reagents in the osteogenic medium. For example, endogenous BMP-6 can be induced by both dexamethasone and vitamin D_3, whereas BMP-2 is only detected in the presence of vitamin D_3.[38] These results suggest that the different active agents in osteogenic medium induce the osteogenic differentiation of ASCs through regulating BMPs and their signaling pathways.

In 1999, Mundy *et al.*, for the first time, reported that statins could effectively stimulate bone formation in rodents, both *in vitro* and *in vivo*.[63] Many subsequent studies confirmed that statins exert their osteoinductive effects through promoting BMP-2 expression.[64] Among statins, simvastatin, an inhibitor of the competitive 3-hydroxy-3-methyl coenzyme A reductase, is considered to be the most potent

inducer for the osteogenic differentiation of mesenchymal stem cells. We recently reported that simvastatin can enhance the osteogenesis of human ASCs *in vitro* and *in vivo* by signiﬁantly increasing the expression of mRNA encoding BMP-2, RUNX2, VEGF, and ﬁbroblast growth factor-2. [3] The upregulated BMP-2 seems to be one of the major mechanisms for the promoting effect of simvastatin on the osteogenesis of human ASCs.[3] In ASCs, endogenous BMPs can also be induced by TGF-b1,[65] sonic hedgehog (shh),[66] inhibitor of b-catenin and TCF-4 (ICAT),[67] and sesamin.[68] These active

commit a certain differentiation direction of ASCs or not. It seemed that, in the most commonly used concentration range (5?50 ng/mL) and without additional osteogenic agents, BMPs cannot signiﬁcantly commit ASCs to either osteogenesis or adipogenesis. One hypothesis is that BMP alone can activate and induce equivalent levels of osteogenic and adipogenic signaling. Both signaling antagonize each other through different signaling levels. The mutual suppression and inhibition between these two signaling result in a noncommitment stage.

The balance between osteogenic and adipogenic signaling can be disequilibrated by the addition of osteogenic agents, such as osteogenic medium and RA. The disequilibration may be mediated, at least partially, by three main switches: endogenous Wnt5a and ERK and changing the ratio of BMPR-IB/BMPR-IA (Fig. 3).

BMPR-IA and BMPR-IB exhibited versatile and divergent effects on the process of osteogenesis both *in vitro* and *in vivo*.[75?7] Their exact functions are highly dependent on factors including cell type and differentiation stage.[75] For mouse ASCs, signaling through BMPR-IB played a more signiﬁcant role in osteogenic differentiation, whereas signaling through BMPR-IA seemed to be more relatively relevant to adipogenesis.[43] Data from 2T3 mouse calvarial stem cells corroborated the distinct functions of these two BMP receptors.[77] Therefore, the type of receptor and level of expression on stem cells can be a switch for their commitment. It is also important to note that RA signaling is indispensable for osteogenic differentiation in mouse ASCs,[43] although this is not the case for human ASCs.[78] Consequently, precautions must be taken when extrapolating data from mouse ASCs to humans.

ERK also appears to be a key switch of adipogenic and osteogenic differentiation of mesenchymal stem cells.[79] Consistent with this ﬁnding, ERK is also important for the adipogenic and osteogenic commitment in ASCs.[68,80?3] Inhibition of ERK by PD98059 blocks the expression of osteogenic differentiation-related proteins in a dose-dependent manner and switches ASCs to adipogenic differentiation.[83] Further, ERK is indispensable for the effects of sesamin,[81] akermanite,[68] wedelolactone,[82] and oncostatin M[80] in promoting the osteogenic differentiation and inhibiting adipogenic differentiation of ASCs. However, how ERK is modulated during osteogenesis of ASCs remains to be elucidated.

Wnt signaling also has an important role in the osteogenic commitment of mesenchymal stem cells (Fig. 3). Wnt5a, a noncanonical Wnt ligand, promotes the osteogenesis by repressing PPAR-g transactivation through CaMKII-TAK1/TAB2-NLK signaling cascade and the subsequent activation of the histone methyltransferase, SETDB1 (SET domain bifurcated 1).[84,85] SETDB1 leads to the formation of a corepressor complex that inactivates PPAR-g function through histone H3-K9 methylation. Thus, noncanonical Wnt5a has emerged as a fate determinant of mesenchymal stem cells through shifting from adipogenesis to osteogenesis.[84,85] Exogenous Wnt5a induces osteogenic differentiation and downregulates PPAR-g expression in human ASCs.[86] However, the interaction between endogenous Wnt and BMP signaling during the osteogenesis of ASCs needs further elucidation.

In contrast with their ambiguous effects *in vitro*, BMPs have a more deﬁnite effect on promoting the *in vivo*

FIG. 3. Schematic diagram depicting the signaling pathways of BMPs in ASCs and the main switches for the osteogenic commitment of ASCs. BMPR-IA, BMP receptor type-IA; BMPR-IB, BMP receptor type-IB; BMPR-II, BMP receptor type-II; CaMK-II, calcium/calmodulin-dependent kinase II; ERK, extracellular signal-regulated kinase; LPL, lipoprotein lipase; NF-kB, nuclear factor kB; NLK, Nemo-like kinase; PPAR-g, peroxisome proliferator-activated receptor gamma; RUNX2, Runt-related transcription factor 2; TAB2, TGF-b-activated kinase 1/MAP3K7-binding protein 2; TAK1, transforming growth factor b-activated kinase-1; TAZ, transcriptional coactivator with PDZ-binding motif; TNF-a, tumor necrosis factor-alpha. /, promotion; x, inhibition. Color images available online at www.liebertpub.com/teb

osteogenesis of ASCs. Endogenous cytokines that are elevated during acute inflammation may also significantly facilitate the osteogenic commitment of ASCs *in vivo* (Fig. 3). Tumor necrosis factor-alpha (TNF-a) is one of the main cytokines responsible for acute inflammation. [87] TNF-a activates nuclear factor-kB (NF-kB), which inhibits the transactivation of PPAR-g through a physical association.[85] NF-kB activation also leads to upregulation of TAZ (transcriptional coactivator with PDZ-binding motif), a coactivator of RUNX2-dependent transcription, and suppression of PPAR-g-dependent transcription.[88] Through these three pathways, TNF-a can help to commit the osteogenic differentiation of ASCs in *in vivo* microenvironments. The role of TNF-a in their osteogenic differentiation is supported by the finding that ASCs can be used to heal acute, but not chronic, calvarial defects in nude mice.[89] TNF-a that occurs during acute inflammation may help to commit the osteogenesis of ASCs, leading to a much more definite effect of exogenous BMPs in inducing the osteogenesis of ASCs *in vivo* than that *in vitro*.

The roles of BMP signaling in the promotion of osteogenic differentiation of ASCs

BMP signaling plays a crucial role in the osteogenesis of ASCs. The upregulation of BMP signaling significantly enhances and accelerates the process. This is supported by the fact that the osteogenesis of ASCs positively correlates to the expression levels of BMPR-II, Smad-1/5, RUNX2, and osterix.

promoted osteogenic differentiation of tendon-derived stem cells. J Orthop Res 29, 390, 2011.
17. Xiang, L., Liang, C., Zhen-Yong, K., Liang-Jun, Y., and Zhong-Liang, D. BMP9-induced osteogenetic differentiation and bone formation of muscle-derived stem cells. J Biomed Biotechnol 2012, 610952, 2012.
18. Reddi, A.H. BMPs: from bone morphogenetic proteins to body morphogenetic proteins. Cytokine Growth Factor Rev 16, 249, 2005.
19. Reddi, A.H., and Reddi, A. Bone morphogenetic proteins (BMPs): from morphogens to metabologens. Cytokine Growth Factor Rev 20, 341, 2009.
20. Tseng, Y.H., Kokkotou, E., Schulz, T.J., Huang, T.L., Winnay, J.N., Taniguchi, C.M., Tran, T.T., Suzuki, R., Espinoza, D.O., Yamamoto, Y., Ahrens, M.J., Dudley, A.T., Norris, A.W., Kulkarni, R.N., and Kahn, C.R. New role of bone morphogenetic protein 7 in brown adipogenesis and energy expenditure. Nature 454, 1000, 2008.
21. Kim, H.J., and Im, G.I. Combination of transforming growth factor-beta2 and bone morphogenetic protein 7 enhances chondrogenesis from adipose tissue-derived mesenchymal stem cells. Tissue Eng Part A 15, 1543, 2009.
22. Derynck, R., and Zhang, Y.E. Smad-dependent and Smad-independent pathways in TGF-beta family signalling. Nature 425, 577, 2003.
23. Guo, J., and Wu, G. The signaling and functions of heterodimeric bone morphogenetic proteins. Cytokine Growth Factor Rev 23, 61, 2012.
24. Xiao, G., Gopalakrishnan, R., Jiang, D., Reith, E., Benson, M.D., and Franceschi, R.T. Bone morphogenetic proteins, extracellular matrix, and mitogen-activated protein kinase signaling pathways are required for osteoblast-specific gene expression and differentiation in MC3T3-E1 cells. J Bone Miner Res 17, 101, 2002.
25. Hata, K., Nishimura, R., Ikeda, F., Yamashita, K., Matsubara, T., Nokubi, T., and Yoneda, T. Differential roles of Smad1 and p38 kinase in regulation of peroxisome proliferator-activating receptor gamma during bone morphogenetic protein 2-induced adipogenesis. Mol Biol Cell 14, 545, 2003.
26. Huang, H., Song, T.J., Li, X., Hu, L., He, Q., Liu, M., Lane, M.D., and Tang, Q.Q. BMP signaling pathway is required for commitment of C3H10T1/2 pluripotent stem cells to the adipocyte lineage. Proc Natl Acad Sci U S A 106, 12670, 2009.
27. Mizrahi, O., Sheyn, D., Tawackoli, W., Kallai, I., Oh, A., Su, S., Da, X., Zarrini, P., Cook-Wiens, G., Gazit, D., and Gazit, Z. BMP-6 is more efficient in bone formation than BMP-2 when overexpressed in mesenchymal stem cells. Gene Ther 20, 370, 2013.
28. Zeng, Q., Li, X., Beck, G., Balian, G., and Shen, F.H. Growth and differentiation factor-5 (GDF-5) stimulates osteogenic differentiation and increases vascular endothelial growth factor (VEGF) levels in fat-derived stromal cells in vitro. Bone 40, 374, 2007.
29. Knippenberg, M., Helder, M.N., Zandieh, D.B., Wuisman, P.I., and Klein-Nulend, J. Osteogenesis versus chondrogenesis by BMP-2 and BMP-7 in adipose stem cells. Biochem Biophys Res Commun 342, 902, 2006.
30. Al-Salleeh, F., Beatty, M.W., Reinhardt, R.A., Petro, T.M., and Crouch, L. Human osteogenic protein-1 induces osteogenic differentiation of adipose-derived stem cells harvested from mice. Arch Oral Biol 53, 928, 2008.
31. Zheng, Y., Wu, G., Zhao, J., Wang, L., Sun, P., and Gu, Z. rhBMP2/7 heterodimer: an osteoblastogenesis inducer of not higher potency but lower effective concentration compared with rhBMP2 and rhBMP7 homodimers. Tissue Eng Part A 16, 879, 2010.
32. Lin, L., Fu, X., Zhang, X., Chen, L.X., Zhang, J.Y., Yu, C.L., Ma, K.T., and Zhou, C.Y. Rat adipose-derived stromal cells expressing BMP4 induce ectopic bone formation in vitro and in vivo. Acta Pharmacol Sin 27, 1608, 2006.
33. Lin, L., Shen, Q., Wei, X., Hou, Y., Xue, T., Fu, X., Duan, X., and Yu, C. Comparison of osteogenic potentials of BMP4 transduced stem cells from autologous bone marrow and fat tissue in a rabbit model of calvarial defects. Calcif Tissue Int 85, 55, 2009.
34. Vicente, L.M.A., Vazquez, G.M.N., Entrena, A., Olmedillas, L.S., Garcia-Arranz, M., Garcia-Olmo, D., and Zapata, A. Low doses of bone morphogenetic protein 4 increase the survival of human adipose-derived stem cells maintaining their stemness and multipotency. Stem Cells Dev 20, 1011, 2011.
35. Mehrkens, A., Saxer, F., Guven, S., Hoffmann, W., Muller, A.M., Jakob, M., Weber, F.E., Martin, I., and Scherberich, A. Intraoperative engineering of osteogenic grafts combining freshly harvested, human adipose-derived cells and physiological doses of bone morphogenetic protein-2. Eur Cell Mater 24, 308, 2012.
36. Shiraishi, T., Sumita, Y., Wakamastu, Y., Nagai, K., and Asahina, I. Formation of engineered bone with adipose stromal cells from buccal fat pad. J Dent Res 91, 592, 2012.
37. Panetta, N.J., Gupta, D.M., Lee, J.K., Wan, D.C., Commons, G.W., and Longaker, M.T. Human adipose-derived stromal cells respond to and elaborate bone morphogenetic protein-2 during in vitro osteogenic differentiation. Plast Reconstr Surg 125, 483, 2010.
38. Zhou, Y.S., Liu, Y.S., and Tan, J.G. Is 1, 25-dihydroxyvitamin D3 an ideal substitute for dexamethasone for inducing osteogenic differentiation of human adipose tissue-derived stromal cells in vitro? Chin Med J (Engl) 119, 1278, 2006.
39. Malladi, P., Xu, Y., Yang, G.P., and Longaker, M.T. Functions of vitamin D, retinoic acid, and dexamethasone in mouse adipose-derived mesenchymal cells. Tissue Eng 12, 2031, 2006.
40. Zuk, P.A., Zhu, M., Ashjian, P., De Ugarte, D.A., Huang, J.I., Mizuno, H., Alfonso, Z.C., Fraser, J.K., Benhaim, P., and Hedrick, M.H. Human adipose tissue is a source of multipotent stem cells. Mol Biol Cell 13, 4279, 2002.
41. Beck, G.R., Jr., Zerler, B., and Moran, E. Phosphate is a specific signal for induction of osteopontin gene expression. Proc Natl Acad Sci U S A 97, 8352, 2000.
42. Song, I., Kim, B.S., Kim, C.S., and Im, G.I. Effects of BMP-2 and vitamin D3 on the osteogenic differentiation of adipose stem cells. Biochem Biophys Res Commun 408, 126, 2011.
43. Wan, D.C., Shi, Y.Y., Nacamuli, R.P., Quarto, N., Lyons, K.M., and Longaker, M.T. Osteogenic differentiation of mouse adipose-derived adult stromal cells requires retinoic acid and bone morphogenetic protein receptor type IB signaling. Proc Natl Acad Sci U S A 103, 12335, 2006.
44. Levi, B., James, A.W., Nelson, E.R., Vistnes, D., Wu, B., Lee, M., Gupta, A., and Longaker, M.T. Human adipose derived stromal cells heal critical size mouse calvarial defects. PLoS One 5, e11177, 2010.
45. E, L.L., Xu, L.L., Wu, X., Wang, D.S., Lv, Y., Wang, J.Z., and Liu, H.C. The interactions between rat-adipose-derived stromal cells, recombinant human bone morphogenetic protein-2, and beta-tricalcium phosphate play an important role in bone tissue engineering. Tissue Eng Part A 16, 2927, 2010.

46. Szpalski, C., Nguyen, P.D., Cretiu, V.C.E., Chesnoiu-Matei, I., Ricci, J.L., Clark, E., Smay, J.E., and Warren, S.M. Bony engineering using time-release porous scaffolds to provide sustained growth factor delivery. J Craniofac Surg 23, 638, 2012.
47. Kang, S.W., Kim, J.S., Park, K.S., Cha, B.H., Shim, J.H., Kim, J.Y., Cho, D.W., Rhie, J.W., and Lee, S.H. Surface modification with fibrin/hyaluronic acid hydrogel on solid-free form-based scaffolds followed by BMP-2 loading to enhance bone regeneration. Bone 48, 298, 2011.
48. Weinand, C., Nabili, A., Khumar, M., Dunn, J.R., Ramella-Roman, J., Jeng, J.C., Jordan, M.H., and Tabata, Y. Factors of osteogenesis influencing various human stem cells on third-generation gelatin/beta-tricalcium phosphate scaffold material. Rejuvenation Res 14, 185, 2011.
49. Jeon, O., Rhie, J.W., Kwon, I.K., Kim, J.H., Kim, B.S., and Lee, S.H. In vivo bone formation following transplantation of human adipose-derived stromal cells that are not differentiated osteogenically. Tissue Eng Part A 14, 1285, 2008.
50. Cowan, C.M., Shi, Y.Y., Aalami, O.O., Chou, Y.F., Mari, C., Thomas, R., Quarto, N., Contag, C.H., Wu, B., and Longaker, M.T. Adipose-derived adult stromal cells heal critical-size mouse calvarial defects. Nat Biotechnol 22, 560, 2004.
51. Dragoo, J.L., Choi, J.Y., Lieberman, J.R., Huang, J., Zuk, P.A., Zhang, J., Hedrick, M.H., and Benhaim, P. Bone induction by BMP-2 transduced stem cells derived from human fat. J Orthop Res 21, 622, 2003.
52. Dragoo, J.L., Samimi, B., Zhu, M., Hame, S.L., Thomas, B.J., Lieberman, J.R., Hedrick, M.H., and Benhaim, P. Tissue-engineered cartilage and bone using stem cells from human infrapatellar fat pads. J Bone Joint Surg Br 85, 740, 2003.
53. Dragoo, J.L., Lieberman, J.R., Lee, R.S., Deugarte, D.A., Lee, Y., Zuk, P.A., Hedrick, M.H., and Benhaim, P. Tissue-engineered bone from BMP-2-transduced stem cells derived from human fat. Plast Reconstr Surg 115, 1665, 2005.
54. Peterson, B., Zhang, J., Iglesias, R., Kabo, M., Hedrick, M., Benhaim, P., and Lieberman, J.R. Healing of critically sized femoral defects, using genetically modified mesenchymal stem cells from human adipose tissue. Tissue Eng 11, 120, 2005.
55. Lin, Y., Tang, W., Wu, L., Jing, W., Li, X., Wu, Y., Liu, L., Long, J., and Tian, W. Bone regeneration by BMP-2 enhanced adipose stem cells loading on alginate gel. Histochem Cell Biol 129, 203, 2008.
56. Hsu, W.K., Wang, J.C., Liu, N.Q., Krenek, L., Zuk, P.A., Hedrick, M.H., Benhaim, P., and Lieberman, J.R. Stem cells from human fat as cellular delivery vehicles in an athymic rat posterolateral spine fusion model. J Bone Joint Surg Am 90, 1043, 2008.
57. Lee, S.J., Kang, S.W., Do, H.J., Han, I., Shin, D.A., Kim, J.H., and Lee, S.H. Enhancement of bone regeneration by gene delivery of BMP2/Runx2 bicistronic vector into adipose-derived stromal cells. Biomaterials 31, 5652, 2010.
58. Lin, C.Y., Lin, K.J., Kao, C.Y., Chen, M.C., Lo, W.H., Yen, T.C., Chang, Y.H., and Hu, Y.C. The role of adipose-derived stem cells engineered with the persistently expressing hybrid baculovirus in the healing of massive bone defects. Biomaterials 32, 6505, 2011.
59. Yang, M., Ma, Q.J., Dang, G.T., Ma, K., Chen, P., and Zhou, C.Y. In vitro and in vivo induction of bone formation based on ex vivo gene therapy using rat adipose-derived adult stem cells expressing BMP-7. Cytotherapy 7, 273, 2005.
60. Kang, Y., Liao, W.M., Yuan, Z.H., Sheng, P.Y., Zhang, L.J., Yuan, X.W., and Lei, L. In vitro and in vivo induction of bone formation based on adeno-associated virus-mediated BMP-7 gene therapy using human adipose-derived mesenchymal stem cells. Acta Pharmacol Sin 28, 839, 2007.
61. Levi, B., Nelson, E.R., Hyun, J.S., Glotzbach, J.P., Li, S., Nauta, A., Montoro, D.T., Lee, M., Commons, G.C., Hu, S., Wu, J.C., Gurtner, G.C., and Longaker, M.T. Enhancement of human adipose-derived stromal cell angiogenesis through knockdown of a BMP-2 inhibitor. Plast Reconstr Surg 129, 53, 2012.
62. Ramasubramanian, A., Shiigi, S., Lee, G.K., and Yang, F. Non-viral delivery of inductive and suppressive genes to adipose-derived stem cells for osteogenic differentiation. Pharm Res 28, 1328, 2011.
63. Mundy, G., Garrett, R., Harris, S., Chan, J., Chen, D., Rossini, G., Boyce, B., Zhao, M., and Gutierrez, G. Stimulation of bone formation in vitro and in rodents by statins. Science 286, 1946, 1999.
64. Maeda, T., Matsunuma, A., Kawane, T., and Horiuchi, N. Simvastatin promotes osteoblast differentiation and mineralization in MC3T3-E1 cells. Biochem Biophys Res Commun 280, 874, 2001.
65. Levi, B., James, A.W., Xu, Y., Commons, G.W., and Longaker, M.T. Divergent modulation of adipose-derived stromal cell differentiation by TGF-beta1 based on species of derivation. Plast Reconstr Surg 126, 412, 2010.
66. James, A.W., Leucht, P., Levi, B., Carre, A.L., Xu, Y., Helms, J.A., and Longaker, M.T. Sonic Hedgehog influences the balance of osteogenesis and adipogenesis in mouse adipose-derived stromal cells. Tissue Eng Part A 16, 2605, 2010.
67. Kim, Y.J., Kim, J.T., Bae, Y.C., Suh, K.T., and Jung, J.S. ICAT participates in proliferation and osteogenic differentiation of human adipose tissue-derived mesenchymal stem cell. Life Sci 83, 851, 2008.
68. Wanachewin, O., Boonmaleerat, K., Pothacharoen, P., Reutrakul, V., and Kongtawelert, P. Sesamin stimulates osteoblast differentiation through p38 and ERK1/2 MAPK signaling pathways. BMC Complement Altern Med 12, 71, 2012.
69. Yang, X., Gong, P., Lin, Y., Zhang, L., Li, X., Yuan, Q., Tan, Z., Wang, Y., Man, Y., and Tang, H. Cyclic tensile stretch modulates osteogenic differentiation of adipose-derived stem cells via the BMP-2 pathway. Arch Med Sci 6, 152, 2010.
70. Kang, K.S., Hong, J.M., Seol, Y.J., Rhie, J.W., Jeong, Y.H., and Cho, D.W. Short-term evaluation of electromagnetic field pretreatment of adipose-derived stem cells to improve bone healing. J Tissue Eng Regen Med 2012 [Epub ahead of print]; DOI: 10.1002/term.1664.
71. Rosen, E.D., and MacDougald, O.A. Adipocyte differentiation from the inside out. Nat Rev Mol Cell Biol 7, 885, 2006.
72. Jeon, M.J., Kim, J.A., Kwon, S.H., Kim, S.W., Park, K.S., Park, S.W., Kim, S.Y., and Shin, C.S. Activation of peroxisome proliferator-activated receptor-gamma inhibits the Runx2-mediated transcription of osteocalcin in osteoblasts. J Biol Chem 278, 23270, 2003.
73. Akune, T., Ohba, S., Kamekura, S., Yamaguchi, M., Chung, U.I., Kubota, N., Terauchi, Y., Harada, Y., Azuma, Y., Nakamura, K., Kadowaki, T., and Kawaguchi, H. PPARgamma insufficiency enhances osteogenesis through osteoblast formation from bone marrow progenitors. J Clin Invest 113, 846, 2004.
74. Lin, Y.F., Jing, W., Wu, L., Li, X.Y., Wu, Y., Liu, L., Tang, W., Long, J., Tian, W.D., and Mo, X.M. Identification of osteo-adipo progenitor cells in fat tissue. Cell Prolif 41, 803, 2008.

75. Mishina, Y., Starbuck, M.W., Gentile, M.A., Fukuda, T., Kasparcova, V., Seedor, J.G., Hanks, M.C., Amling, M., Pinero, G.J., Harada, S., and Behringer, R.R. Bone morphogenetic protein type IA receptor signaling regulates postnatal osteoblast function and bone remodeling. J Biol Chem 279, 27560, 2004.
76. Kaps, C., Hoffmann, A., Zilberman, Y., Pelled, G., Haupl, T., Sittinger, M., Burmester, G., Gazit, D., and Gross, G. Distinct roles of BMP receptors type IA and IB in osteo-/chondrogenic differentiation in mesenchymal progenitors (C3H10T1/2). Biofactors 20, 71, 2004.
77. Chen, D., Ji, X., Harris, M.A., Feng, J.Q., Karsenty, G., Celeste, A.J., Rosen, V., Mundy, G.R., and Harris, S.E. Differential roles for bone morphogenetic protein (BMP) receptor type IB and IA in differentiation and specification of mesenchymal precursor cells to osteoblast and adipocyte lineages. J Cell Biol 142, 295, 1998.
78. Levi, B., and Longaker, M.T. Concise review: adipose-derived stromal cells for skeletal regenerative medicine. Stem Cells 29, 576, 2011.
79. Jaiswal, R.K., Jaiswal, N., Bruder, S.P., Mbalaviele, G., Marshak, D.R., and Pittenger, M.F. Adult human mesenchymal stem cell differentiation to the osteogenic or adipogenic lineage is regulated by mitogen-activated protein kinase. J Biol Chem 275, 9645, 2000.
80. Song, H.Y., Jeon, E.S., Kim, J.I., Jung, J.S., and Kim, J.H. Oncostatin M promotes osteogenesis and suppresses adipogenic differentiation of human adipose tissue-derived mesenchymal stem cells. J Cell Biochem 101, 1238, 2007.
81. Gu, H., Guo, F., Zhou, X., Gong, L., Zhang, Y., Zhai, W., Chen, L., Cen, L., Yin, S., Chang, J., and Cui, L. The stimulation of osteogenic differentiation of human adipose-derived stem cells by ionic products from akermanite dissolution via activation of the ERK pathway. Biomaterials 32, 7023, 2011.
82. Lim, S., Jang, H.J., Park, E.H., Kim, J.K., Kim, J.M., Kim, E.K., Yea, K., Kim, Y.H., Lee-Kwon, W., Ryu, S.H., and Suh, P.G. Wedelolactone inhibits adipogenesis through the ERK pathway in human adipose tissue-derived mesenchymal stem cells. J Cell Biochem 113, 3436, 2012.
83. Liu, Q., Cen, L., Zhou, H., Yin, S., Liu, G., Liu, W., Cao, Y., and Cui, L. The role of the extracellular signal-related kinase signaling pathway in osteogenic differentiation of human adipose-derived stem cells and in adipogenic transition initiated by dexamethasone. Tissue Eng Part A 15, 3487, 2009.
84. Takada, I., Mihara, M., Suzawa, M., Ohtake, F., Kobayashi, S., Igarashi, M., Youn, M.Y., Takeyama, K., Nakamura, T., Mezaki, Y., Takezawa, S., Yogiashi, Y., Kitagawa, H., Yamada, G., Takada, S., Minami, Y., Shibuya, H., Matsumoto, K., and Kato, S. A histone lysine methyltransferase activated by non-canonical Wnt signalling suppresses PPAR-gamma transactivation. Nat Cell Biol 9, 1273, 2007.
85. Takada, I., Kouzmenko, A.P., and Kato, S. Molecular switching of osteoblastogenesis versus adipogenesis: implications for targeted therapies. Expert Opin Ther Targets 13, 593, 2009.
86. Santos, A., Bakker, A.D., de Blieck-Hogervorst, J.M., and Klein-Nulend, J. WNT5A induces osteogenic differentiation of human adipose stem cells via rho-associated kinase ROCK. Cytotherapy 12, 924, 2010.
87. Locksley, R.M., Killeen, N., and Lenardo, M.J. The TNF and TNF receptor superfamilies: integrating mammalian biology. Cell 104, 487, 2001.
88. Cho, H.H., Shin, K.K., Kim, Y.J., Song, J.S., Kim, J.M., Bae, Y.C., Kim, C.D., and Jung, J.S. NF-kappaB activation stimulates osteogenic differentiation of mesenchymal stem cells derived from human adipose tissue by increasing TAZ expression. J Cell Physiol 223, 168, 2010.
89. Levi, B., James, A.W., Nelson, E.R., Peng, M., Wan, D.C., Commons, G.W., Lee, M., Wu, B., and Longaker, M.T. Acute skeletal injury is necessary for human adipose-derived stromal cell-mediated calvarial regeneration. Plast Reconstr Surg 127, 1118, 2011.
90. Zeng, Y., Qu, X., Li, H., Huang, S., Wang, S., Xu, Q., Lin, R., Han, Q., Li, J., and Zhao, R.C. MicroRNA-100 regulates osteogenic differentiation of human adipose-derived mesenchymal stem cells by targeting BMPR2. FEBS Lett 586, 2375, 2012.
91. Zhang, X., Yang, M., Lin, L., Chen, P., Ma, K.T., Zhou, C.Y., and Ao, Y.F. Runx2 overexpression enhances osteoblastic differentiation and mineralization in adipose-derived stem cells *in vitro* and *in vivo*. Calcif Tissue Int 79, 169, 2006.
92. Levi, B., Nelson, E.R., Li, S., James, A.W., Hyun, J.S., Montoro, D.T., Lee, M., Glotzbach, J.P., Commons, G.W., and Longaker, M.T. Dura mater stimulates human adipose-derived stromal cells to undergo bone formation in mouse calvarial defects. Stem Cells 29, 1241, 2011.
93. Wu, L., Wu, Y., Lin, Y., Jing, W., Nie, X., Qiao, J., Liu, L., Tang, W., and Tian, W. Osteogenic differentiation of adipose derived stem cells promoted by overexpression of osterix. Mol Cell Biochem 301, 83, 2007.
94. Lee, J.S., Lee, J.M., and Im, G.I. Electroporation-mediated transfer of Runx2 and Osterix genes to enhance osteogenesis of adipose stem cells. Biomaterials 32, 760, 2011.
95. Luzi, E., Marini, F., Sala, S.C., Tognarini, I., Galli, G., and Brandi, M.L. Osteogenic differentiation of human adipose tissue-derived stem cells is modulated by the miR-26a targeting of the SMAD1 transcription factor. J Bone Miner Res 23, 287, 2008.
96. Chen, P.Y., Sun, J.S., Tsuang, Y.H., Chen, M.H., Weng, P.W., and Lin, F.H. Simvastatin promotes osteoblast viability and differentiation via Ras/Smad/Erk/BMP-2 signaling pathway. Nutr Res 30, 191, 2010.

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