Genes & Diseases (2018) 5, 172–184

Available online at www.sciencedirect.com

ScienceDirect

journal homepage: http://ees.elsevier.com/gendis/default.asp

Characterization of the essential role of bone morphogenetic protein 9 (BMP9) in osteogenic differentiation of mesenchymal stem cells (MSCs) through RNA interference

Shujuan Yan [a,b], Ruyi Zhang [a,b], Ke Wu [b,c], Jing Cui [a,b], Shifeng Huang [b,c], Xiaojuan Ji [b,c], Liping An [b,d], Chengfu Yuan [b,e], Cheng Gong [b,f], Linghuan Zhang [b,c], Wei Liu [b,c], Yixiao Feng [b,c], Bo Zhang [b,d], Zhengyu Dai [b,g], Yi Shen [b,h], Xi Wang [a,b], Wenping Luo [b,c], Bo Liu [b,c], Rex C. Haydon [b], Michael J. Lee [b], Russell R. Reid [b,i], Jennifer Moriatis Wolf [b], Qiong Shi [a,b], Hue H. Luu [b], Tong-Chuan He [b], Yaguang Weng [a,*]

[a] *Ministry of Education Key Laboratory of Diagnostic Medicine and School of Laboratory Medicine, 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] *The School of Pharmacy and the Affiliated Hospitals of Chongqing Medical University, Chongqing 400016, China*
[d] *Key Laboratory of Orthopaedic Surgery of Gansu Province and the Department of Orthopaedic Surgery, The Second Hospital of Lanzhou University, Lanzhou, 730030, China*
[e] *Department of Biochemistry and Molecular Biology, China Three Gorges University School of Medicine, Yichang 443002, China*
[f] *Department of Surgery, The Affiliated Zhongnan Hospital of Wuhan University, Wuhan 430071, China*
[g] *Department of Orthopaedic Surgery, Chongqing Hospital of Traditional Chinese Medicine, Chongqing 400021, China*
[h] *Department of Orthopaedic Surgery, Xiangya Second Hospital of Central South University, Changsha 410011, China*
[i] *Department of Surgery, Laboratory of Craniofacial Biology and Development, Section of Plastic Surgery, The University of Chicago Medical Center, Chicago, IL 60637, USA*

Received 6 April 2018; accepted 17 April 2018
Available online 27 April 2018

* *Corresponding author.* School of Clinical Laboratory Medicine, Chongqing Medical University, Chongqing 400016, China.Fax: +86 23 6848 5658.
E-mail address: yaguangweng@126.com (Y. Weng).
Peer review under responsibility of Chongqing Medical University.

https://doi.org/10.1016/j.gendis.2018.04.006

KEYWORDS
BMP9;
Bone formation;
Mesenchymal stem cells;
Osteogenic differentiation;
RNA interference;
Recombinant adenovirus;
siRNA

Abstract Mesenchymal stem cells (MSCs) are multipotent stem cells and capable of differentiating into multiple cell types including osteoblastic, chondrogenic and adipogenic lineages. We previously identified BMP9 as one of the most potent BMPs that induce osteoblastic differentiation of MSCs although exact molecular mechanism through which BMP9 regulates osteogenic differentiation remains to be fully understood. Here, we seek to develop a recombinant adenovirus system to optimally silence mouse BMP9 and then characterize the important role of BMP9 in osteogenic differentiation of MSCs. Using two different siRNA bioinformatic prediction programs, we design five siRNAs targeting mouse BMP9 (or simB9), which are expressed under the control of the converging H1 and U6 promoters in recombinant adenovirus vectors. We demonstrate that two of the five siRNAs, simB9-4 and simB9-7, exhibit the highest efficiency on silencing exogenous mouse BMP9 in MSCs. Furthermore, simB9-4 and simB9-7 act synergistically in inhibiting BMP9-induced expression of osteogenic markers, matrix mineralization and ectopic bone formation from MSCs. Thus, our findings demonstrate the important role of BMP9 in osteogenic differentiation of MSCs. The characterized simB9 siRNAs may be used as an important tool to investigate the molecular mechanism behind BMP9 osteogenic signaling. Our results also indicate that recombinant adenovirus-mediated expression of siRNAs is efficient and sustained, and thus may be used as an effective delivery vehicle of siRNA therapeutics.

Introduction

Mesenchymal stem cells (MSCs) are multipotent stem cells with self-renewal and capability of differentiating into multiple cell types, such as osteoblastic, chondrogenic, and adipogenic lineages.[1–8] MSCs have attracted profound attentions in the arena of stem cell biology and regenerative medicine.[4,7–12] Osteogenic differentiation from MSCs is a tightly regulated process of sequential events that recapitulate most of the molecular processes occurring during bone and skeletal development.[7,8,13] While osteogenic differentiation is regulated by numerous pathways, such as Wnt, Insulin-like growth factors (IGFs), Fibroblast growth factor (FGFs) and Notch,[3,7,8,14–23] bone morphogenetic proteins (BMPs) are considered as the only group of osteoinductive factors that can induce de novo bone formation from MSCs.[24,25]

BMPs are members of the TGF-β superfamily and play critical roles in skeletal development, bone formation and stem cell differentiation.[3,24–26] At least 14 types of BMPs have been identified in humans and rodents.[24,25,27] We conducted a comprehensive analysis of the osteogenic activities of the 14 types of BMPs, and found that BMP9 (also known as growth differentiation factor 2, or GDF2) is one of the most potent BMPs that induce osteoblastic differentiation of MSCs.[24,28–31] However, BMP9 is one of the least studied BMPs, and the exact molecular mechanism through which BMP9 regulates osteogenic differentiation remains to be fully understood.

Originally discovered in *C. elegans* and subsequently demonstrated in diverse eukaryotes, such as insects, plants, fungi and vertebrates, RNA interference (RNAi) is a cellular process of sequence-specific, post-transcriptional gene silencing initiated by short double-stranded RNAs (dsRNA), or short interfering RNAs (siRNAs), which are homologous to the gene being suppressed through the RNA-induced silencing complex (RISC).[32–39] Since its discovery, RNAi has become a valuable and powerful tool to analyze loss-of-function phenotypes,[33–38] as well as offers unprecedented opportunities for developing novel and effective therapeutics for human diseases.[40–45] Nonetheless, the silencing efficiency of siRNAs is highly empirical and varies drastically, depending on many parameters, such as mRNA secondary structures, target availability, status of matching and intrinsic characteristics of mRNA and siRNA.[33,46–49]

In this study, we developed a recombinant adenovirus system to express the optimal siRNAs targeting mouse BMP9 and characterized the important role of BMP9 in osteogenic differentiation of MSCs. Using different siRNA bioinformatic prediction programs, we designed five siRNAs targeting mouse BMP9 (or simB9 siRNAs), which were expressed under the control of converging H1 and U6 promoters in recombinant adenovirus vectors. We demonstrated that two of the five siRNAs, simB9-4 and simB9-7, exhibited the highest efficiency on silencing exogenous mouse BMP9 in MSCs. Furthermore, we demonstrated that simB9-4 and simB9-7 acted synergistically in impairing BMP9-induced expression of osteogenic markers, matrix mineralization and ectopic bone formation from MSCs. Thus, our findings demonstrate the important role of BMP9 in osteogenic differentiation of MSCs. The characterized simB9 siRNAs may be used as an important tool to investigate the molecular mechanism behind BMP9 osteogenic signaling. Lastly, our results indicate that recombinant adenovirus-mediated