Bone 112 (2018) 24–34

Contents lists available at ScienceDirect

Bone

journal homepage: www.elsevier.com/locate/bone

Full Length Article

# Sclerostin vaccination mitigates estrogen deficiency induction of bone mass loss and microstructure deterioration

Feng-Sheng Wang[a,b,c,1], Re-Wen Wu[d,1], Wei-Shiung Lain[a,b], Tsai-Chen Tsai[b], Yu-Shan Chen[a,b], Yi-Chih Sun[a,b], Huei-Jing Ke[a,b], Jui-Chen Li[a,b], Jaulang Hwang[e], Jih-Yang Ko[d,*]

[a] Department of Medical Research, Kaohsiung Chang Gung Memorial Hospital, Kaohsiung, Taiwan
[b] Core Laboratory for Phenomics and Diagonistics, Kaohsiung Chang Gung Memorial Hospital, Kaohsiung, Taiwan
[c] Graduate Institute of Clinical Medical Sciences, Chang Gung University College of Medicine, Kaohsiung Chang Gung Memorial Hospital, Kaohsiung, Taiwan
[d] Department of Orthopedic Surgery, Kaohsiung Chang Gung Memorial Hospital, Kaohsiung, Taiwan
[e] Institute of Molecular Biology, Academia Sinica, Taipei, Taiwan

ARTICLE INFO

*Keywords:*
Osteoporosis
Estrogen deficiency
Sclerostin
Fc fusion protein
Vaccination

ABSTRACT

Sclerostin (SOST) is a Wnt signaling inhibitor detrimental to osteogenic differentiation and bone mineral acquisition. While control of SOST action delays the pathogenesis of skeletal disorders, the effects of SOST vaccination on the estrogen deficiency-induced bone deterioration remain elusive. In this study, we generated a SOST-Fc fusion protein which was composed of a SOST peptide Pro-Asn-Ala-Ile-Gly along with an IgG Fc fragment. SOST-Fc vaccination increased serum anti-SOST antibody levels and reduced serum SOST concentrations in mice. In vitro, anti-SOST serum attenuated the SOST-induced inhibition of osteogenic gene expression in osteoblast cultures. Administration with SOST-Fc increased serum levels of bone formation marker osteocalcin and alleviated the ovariectomy escalation of serum resorption markers CTX-1 and TRAP5b concentrations. It remarkably lessened the estrogen deficiency-mediated deterioration of bone mineral density, morphometric characteristics of trabecular bone, and mechanical strength of femurs and lumbar spines. The SOST-Fc-treated skeletal tissue exhibited moderate responses to the adverse actions of ovariectomy to bone mineral accretion, osteoclast surface, trabecular separation, and fatty marrow histopathology. SOST-Fc treatment increased serum osteoclast-inhibitory factor osteoprotegrin levels in conjunction with strong Wnt3a, β-catenin, and TCF4 immunostaining in osteoblasts, whereas it weakened the estrogen deficiency enhancement of osteoclast-promoting factor receptor activator of nuclear factor-κB ligand. Taken together, blockade of SOST action by SOST-Fc vaccination sustains Wnt signaling, which harmonizes bone mineral accretion and resorption reactions and thereby ameliorates ovariectomy-induced bone loss. This study highlights SOST-Fc fusion protein as a new molecular therapeutic potential for preventing from osteoporotic disorders.

## 1. Introduction

Excessive bone loss aggravated by estrogen deficiency worsens microstructural and mechanical properties of skeletons and ultimately increases the prevalence of osteoporotic fracture and disability [1]. Management of osteoporosis pathogenesis has become a prioritized task for reducing the comorbidities of degenerative skeletal disorders [2]. In addition to vitamin D supplement and bisphosphonate medication [3,4], many biological agents that modulate receptor activator of NFκB ligand [5], vitamin K [6], and parathyroid hormone [7] have been observed to reduce the incidence of postmenopausal osteoporosis and osteoporotic fracture.

Sclerostin (SOST) is found to inhibit biological function of Wnt signal components through targeting Wnt co-receptors low density lipoprotein receptor-related protein (LRP) 5 and 6 [8]. This molecule reduces mineralization reactions of skeletons [9] and calcification activities of vascular and renal tissues [10]. SOST knockout mice exhibit abundant bone mass, trabecular volume, and cortical thickness [11]. Methylation status [12] and polymorphism [13] of SOST gene are linked to the incidence of osteoporosis. Increased serum SOST levels are correlated with the occurrence of osteoporotic fracture in menopausal women [14]. Strong SOST expression in bile duct is associated with the development of biliary cirrhosis-mediated bone disorders [15]. In contrast to the deleterious actions of SOST to skeletal tissue,

* Corresponding author at: Department of Orthopedic Surgery, Kaohsiung Chang Gung Memorial Hospital, 123, Ta-Pei Road, Niao-Sung District, Kaohsiung 83303, Taiwan.
*E-mail addresses:* wangfs@ms33.hinet.net (F.-S. Wang), kojy@cgmh.org.tw (J.-Y. Ko).
[1] FS Wang & RW Wu contribute equally to this article.

https://doi.org/10.1016/j.bone.2018.04.007
Received 26 October 2017; Received in revised form 19 March 2018; Accepted 9 April 2018
Available online 11 April 2018
8756-3282/ 

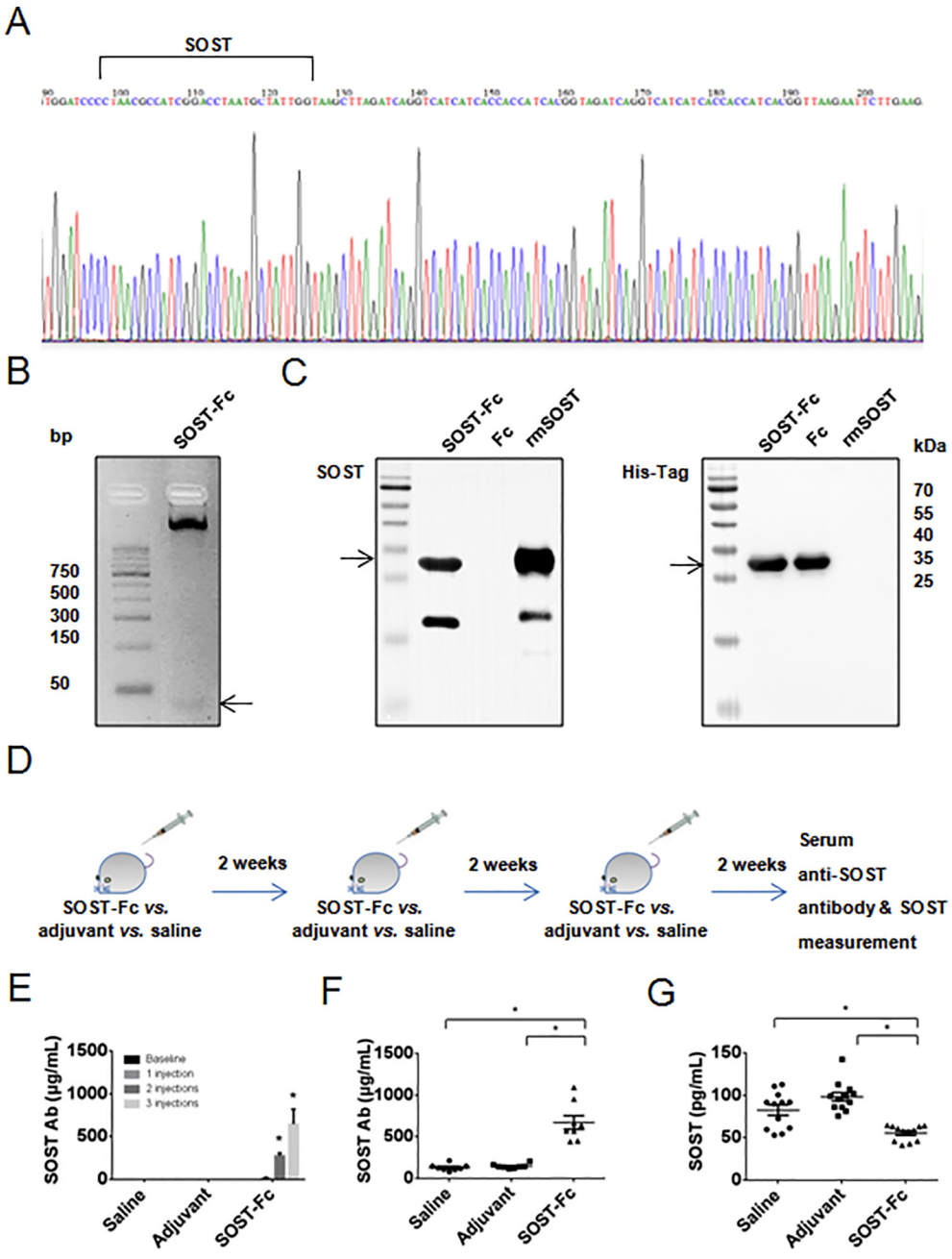


**Fig. 1.** Analyses of SOST-Fc fusion proteins. (*A*) Gene sequencing analysis of the SOST-Fc. (*B*) Agarose electrophoresis of pET-15b expression vector that contained the SOST-Fc sequence. (*C*) Immunoblotting of SOST-Fc fusion protein showing positive SOST immunoreactivity. (*D*) Schematic drawing for SOST-Fc immunization. Mice showed (*E*) a dose response to the SOST-Fc elevation of serum anti-SOST antibody levels, (*F*) an increase in the levels of serum anti-SOST antibody (*G*) and a reduction in serum SOST concentration after 3 injections of 20 μg/kg/injection. * indicates significant difference ($P < 0.05$) between groups analyzed by an ANOVA test and a post hoc test.

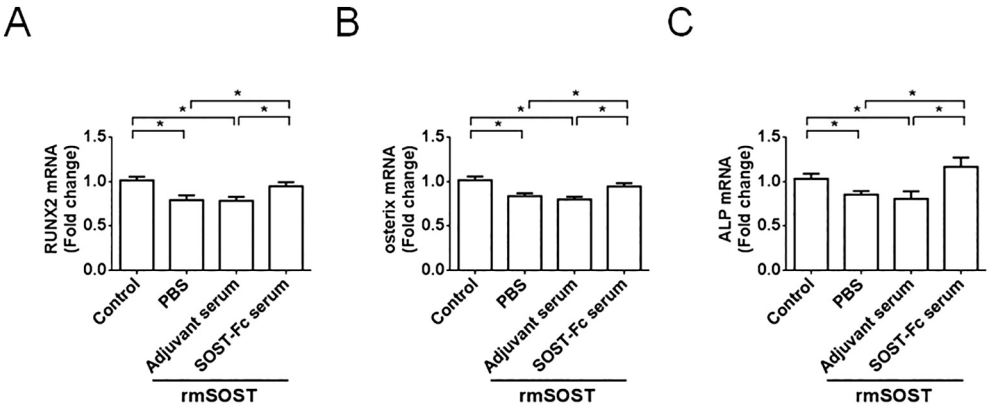


**Fig. 2.** Effects of anti-SOST serum on osteogenic activity of osteoblasts exposed to SOST recombinant protein. The SOST inhibition of the expression of (*A*) Runx2, (*B*) osterix, and (*C*) bone alkaline phosphatase were compromised by anti-SOST serum. Experiments were repeated 5 times. * indicates significant difference ($P < 0.05$) between groups analyzed by an ANOVA test and a post hoc test.