Transgenic expression of *Dspp* partially rescued the long bone defects of *Dmp1*-null mice

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Abstract

Dentin matrix protein 1 (DMP1) and dentin sialophosphoprotein (DSPP) belong to the Small Integrin-Binding Ligand *N*-linked Glycoprotein (SIBLING) family. In addition to the features common to all SIBLING members, DMP1 and DSPP share several unique similarities in chemical structure, proteolytic activation and tissue localization. Mutations in, or deletion of *DMP1*, cause autosomal recessive hypophosphatemic rickets along with dental defects; *DSPP* mutations or its ablation are associated with dentinogenesis imperfecta. While the roles and functional mechanisms of DMP1 in osteogenesis have been extensively studied, those of DSPP in long bones have been studied only to a limited extent. Previous studies by our group revealed that transgenic expression of *Dspp* completely rescued the dentin defects of *Dmp1*-null ($Dmp1^{-/-}$) mice. In this investigation, we assessed the effects of transgenic *Dspp* on osteogenesis by analyzing the formation and mineralization of the long bones in $Dmp1^{-/-}$ mice that expresses a transgene encoding full-length DSPP driven by a 3.6-kb rat *Col1a1* promoter (referred as "$Dmp1^{-/-}$;*Dspp*-Tg mice"). We characterized the long bones of the $Dmp1^{-/-}$;*Dspp*-Tg mice at different ages and compared them with those from $Dmp1^{-/-}$ and $Dmp1^{+/-}$ (normal control) mice. Our analyses showed that the long bones of $Dmp1^{-/-}$;*Dspp*-Tg mice had a significant increase in cortical bone thickness, bone volume and mineral density along with a remarkable restoration of trabecular thickness compared to those of the $Dmp1^{-/-}$ mice. The long bones of $Dmp1^{-/-}$;*Dspp*-Tg mice underwent a dramatic reduction in the amount of osteoid, significant improvement of the collagen fibrillar network, and better organization of the lacunocanalicular system, compared to the $Dmp1^{-/-}$ mice. The elevated levels of biglycan, bone sialoprotein and osteopontin in $Dmp1^{-/-}$ mice were also noticeably corrected by the transgenic expression of *Dspp*. These findings suggest that DSPP and DMP1 may function synergistically within the complex milieus of bone matrices.

Introduction

The organic components in the extracellular matrix (ECM) of bone are composed of collagen type I and a number of non-collagenous proteins (NCPs). One family of NCPs is the Small Integrin-Binding Ligand N-linked Glycoprotein (SIBLING) family, which consists of osteopontin (OPN), bone sialoprotein (BSP), dentin matrix protein 1 (DMP1), dentin sialophosphoprotein (DSPP) and matrix extracellular phosphoglycoprotein (MEPE) [1]. These SIBLING family members play important biological roles in the formation and mineralization of bone and dentin [1–4], as evidenced by the observations that mutations in or ablation of their genes are associated with developmental abnormalities in the two tissues [5–7].

DMP1, first cloned from a rat odontoblast cDNA library, has been identified in dentin, bone and cementum as well as in some non-mineralized tissues [8–10]. In the appendicular skeleton, DMP1 is expressed by osteocytes, osteoblasts and hypertrophic chondrocytes [11–13]. *Dmp1*-deficient mice displayed severe defects in the cartilage and bone, which resembled the manifestations of autosomal recessive hypophosphatemic rickets (ARHR), a human hereditary disease caused by mutations in

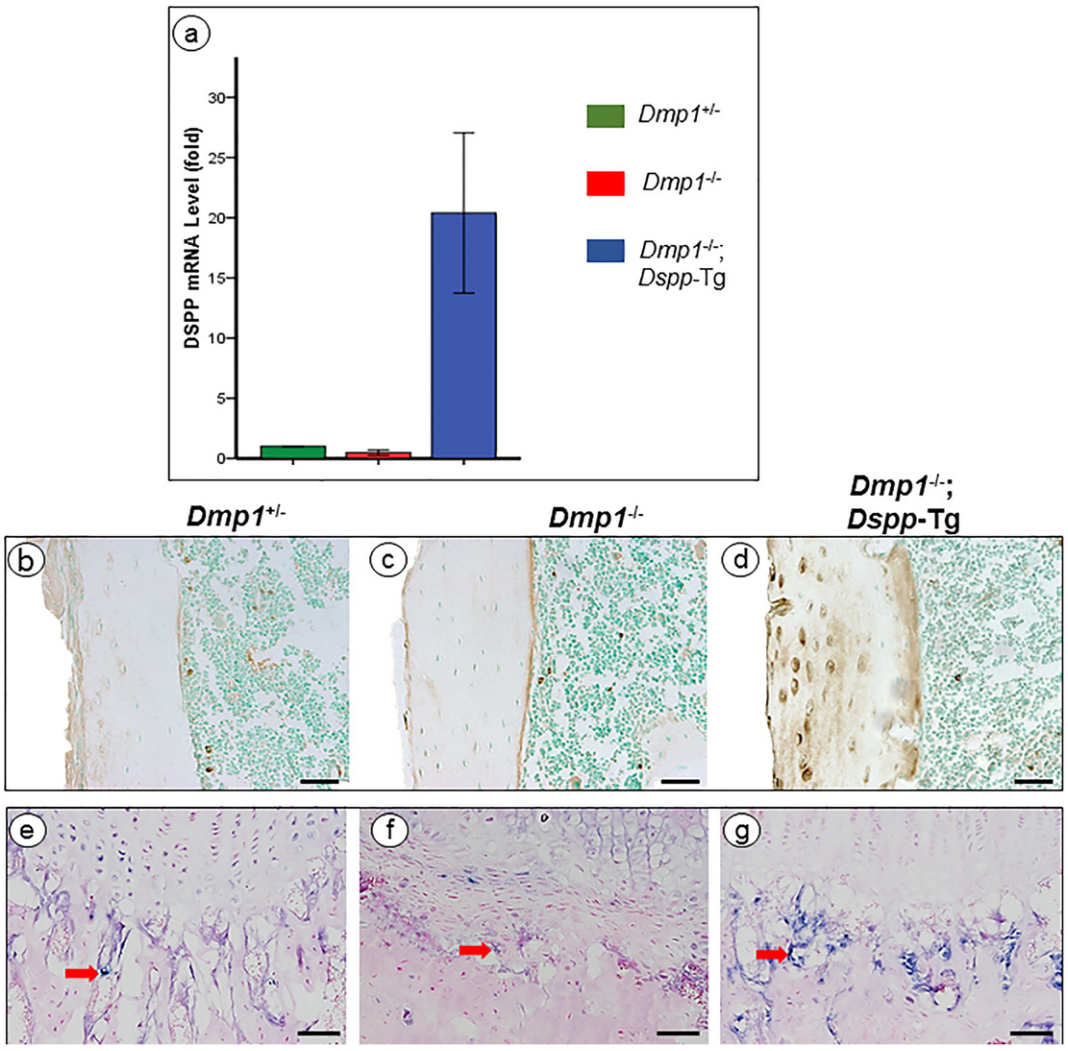

Fig. 1. Expression of *Dspp* transgene. a, RT-qPCR analyses; b-d, immunohistochemistry staining using the anti-DSP antibody; e-g, in situ hybridization analyses for DSPP mRNA. In the RT-qPCR analyses (a), we measured the mRNA levels of DSPP in the femurs of 3-month-old mice from each group. The DSPP mRNA level in the normal control ($Dmp1^{+/-}$) mice (green bar) was taken as 1. DSPP mRNA level in the $Dmp1^{-/-}$ mice (red bar) was 30% of the $Dmp1^{+/-}$ mice, while its level in the $Dmp1^{-/-}$;*Dspp*-Tg mice (blue bar) was 20 fold of the normal. The anti-DSP immunostaining exhibited signals for this protein around the osteocyte lacunae in the cortical bone in the mid-shaft region of the femurs of control mice (b). The anti-DSP signals were slightly weaker in the cortical bone of $Dmp1^{-/-}$ mice (c) than in the $Dmp1^{+/-}$ controls. The cortical bone of $Dmp1^{-/-}$;*Dspp*-Tg mice (d) demonstrated a higher level of anti-DSP immunoreactivity compared to the other two groups of mice. *In situ* hybridization analyses show the presence of DSPP mRNA (arrows) in the newly formed bone proximal to the epiphyseal growth plate of $Dmp1^{+/-}$ mice (e); DSPP mRNA level was lower in $Dmp1^{-/-}$ mice (f) than in the $Dmp1^{+/-}$ mice. In the same region, $Dmp1^{-/-}$;*Dspp*-Tg mice (g) showed elevated level of DSPP mRNA confirming the higher expression levels of *Dspp* transgene in these mice. Scale bar: 50 μm in b-g.

the *DMP1* gene. This condition was characterized by the elevation of serum fibroblast growth factor 23 (FGF23) and a reduction of serum phosphorus, along with malformed and hypomineralized bone and dentin [14,15]. Although DSPP was originally thought to be exclusively expressed by odontoblasts, the dentin-forming cells, later on its expression was detected in bone, cementum and certain non-mineralizing tissues including the salivary glands and kidneys [16–19]. Our previous studies showed that the expression level of DSPP in the rat long bone is approximately 1/400th of that in the rat dentin [19]. Mouse and human genetic studies have associated *DSPP* mutations or its ablation with dentinogenesis imperfecta, characterized by thinner dentin, enlarged pulp chamber and widened predentin [5,6,20,21]. While *Dspp* knockout mice have severe defects in the formation and mineralization of dentin, the changes in the long bones of *Dspp*-deficient mice are mild [22].