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Opinion

Mice expressing DMP1 exhibit impaired skeletal growth

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Abstract

A noncollagenous protein expressed in dentin called Dentin Matrix Protein 1 (DMP1) bone, dentin, and cartilage are examples of mineralized tissues. Transgenic mice that overexpress DMP1 under the control of the CMV gene were used to explore the role of DMP1 during bone development. There were produced promoters. These mice showed a phenotype of enhanced bone mineralization. Additionally, it was discovered that the chondrocytes in the epiphyseal growth plate underwent rapid terminal differentiation. Bone marrow stem cells were stimulated with DMP1 and tested for “early” and “late” markers for osteoblast differentiation in order to look into the potential function of DMP1 in osteoblast differentiation. Within two days of receiving DMP1, CBFA1, BMP2, COL1, and OCN expression levels increased. The ability of bone marrow stem cells to differentiate and create a mineralized matrix in the presence of DMP1 was established by an in vitro mineralized nodule formation test. Together, these findings lend credence to the hypothesis that DMP1 acts as a crucial regulatory molecule necessary for the healthy growth and development of bone and cartilage.

Keywords: Dentin matrix protein, bone marrow, bone mineral density

INTRODUCTION

Bone growth and remodelling are necessary for the complex process of bone creation. The main components of the extracellular matrix in hard tissues are type I collagen and Noncollagenous Proteins (NCPs). Dentin Matrix Protein 1 (DMP1), osteopontin, osteonectin, matrix Gla protein, bone sialoprotein, and osteocalcin are some of the most common NCPs of the bone matrix that serve in osteoblast development and matrix mineralization. A number of these NCPs have an impact on how orientated hydroxyapatite grows both temporally and spatially. A seminal matrix protein known as *DMP1* exists among the NCPs and was initially cloned from a rat cDNA pool.

The molecule has been isolated from osseous tissue, and although the nomenclature suggests that it functions in the dentin matrix, it has also been hypothesised that it also plays a role in the growth and ossification of bone. After the rat *DMP1* gene was cloned, a number of investigations were conducted to characterise the molecule and ascertain its role in the biology of calcified tissues. Understanding the process of osteogenesis and subsequent pathological entities requires defining the process of hard tissue creation.

DMP1 is a 58 kDa phosphoprotein with a pI of 3 that is present in the matrix of bone and dentin. DMP1 expression is seen throughout the ossification process in the bone.

DMP1's ability to nucleate hydroxyapatite was originally demonstrated in *in vitro* investigations, which provided the first proof of the protein's functional involvement in mineralization. The absence of an obvious skeletal or dental phenotype in early development in DMP1-null animals, however, suggests functional redundancy because of overlapping expression with other non-collagenous proteins such as BAG-75. As a result of pathological alterations in bone mineralization and problems in cartilage production, Feng and colleagues have shown that DMP1-null mice have faulty osteocyte maturation.

In the current investigation, we created a Transgenic (TG) mouse that overexpressed *DMP1* to explore the biological function of *DMP1* *in vivo*. Since the Cytomegalovirus (CMV) promoter is thought to be among the most potent and to have the most promiscuous regulatory elements, it was chosen to guide high transgenic expression. This study's goal was to describe the skeletal characteristics of TG mice who were overexpressing *DMP1*.

The rat *DMP1* cDNA's coding region and 3' untranslated region were amplified using Polymerase Chain Reaction (PCR), which is how the CMV-DMP1 plasmid was created. After being digested with Bgl II and Pvu II, this fragment was cloned into the KpnI and XhoI sites of the pcDNA 3.1+ vector (Invitrogen, Carlsbad, CA, USA) to produce the cassette encoding the CMV-DMP1 and bovine growth hormone *poly A* transgene. In mouse blastocysts, the liberated DNA fragment (transgene) was microinjected. At the University of Illinois at Chicago Transgenic Facility, microinjections into CD-1 (Charles River Laboratories, Wilmington, MA, USA) outbred mice were carried out in accordance with institutionally authorised methods. Using the DNeasy tissue kit (Qiagen, Valenica, CA, USA), genomic DNA was isolated from tail pieces to identify the F0 *DMP1*-positive lines. PCR was performed using primers that are specific to CMV. In a nutshell, 100 ng of genomic DNA underwent 35 cycles of PCR at 94°C for 30 sec, 56°C for 30 sec, and 72°C for 30 sec. Utilising the following precise primers for the CMV promoter, genotyping was conducted.

T-PER lysis buffer (Pierce Biotechnology, Rockford, IL, USA) was used to extract the total protein from liver, muscle, and heart tissue samples. The supernatant was recovered after centrifuging 0.1 g of fresh tissue from newborn animals at 15,000 g for 15 minutes in 2 ml of T-PER lysis buffer. With DMP1 antibody that has been affinity-

purified, Western blot analysis was done. Three-day-old animal femurs were homogenised in liquid nitrogen and extracted with 0.5 M EDTA (Ethylenediaminetetraacetic Acid) at pH 7.5 in the presence of a protease inhibitor combination to obtain the total protein from the bone tissue.

At four weeks of age, the TGs (n=5) and the wild type (WT; n=5) were dissected out, and the mandibles were also removed.

After being decalcified, the tissues were paraffin-embedded. Sections were prepared with various histological stains and cut at a thickness of 5 μ m. Masson's trichrome, Safranin O/Fast green, and Hematoxylin and Eosin (H&E) stain were employed to identify the general tissue architecture, connective tissue fibres, and proteoglycans, respectively.

Skeletons were produced in accordance with Tylzanowski and colleagues' instructions.¹⁷ In a nutshell, mice were carbon dioxide gas sacrificed at three days, five days and two weeks postnatally (n=5).

The nonskeletal tissues were digested with 2% potassium hydroxide, then fixed for 72 hours in 95% ethanol, fixed for 24 hours in acetone, and stained for 36 hours with alcian blue. After being cleaned in 95% ethanol, the stained bodies were immersed in a 95% ethanol bath for 48 hours, with a 24 hour change in the solution.

Prior to cleaning and counterstaining, pictures of the caudal vertebral column were obtained using a 5 megapixel Fuji digital camera (Fujifilm, Valhalla, NY, USA). The bodies were next immersed in a 1% potassium hydroxide solution until clarity became apparent, and then counterstained with alizarin red for 24 hours. The specimens were treated with a succession of 2% potassium hydroxide-glycerol solutions every 12 hours in the KOH-glycerol ratios of 80:20, 60:40, 40:60, and 20:80 following secondary cleaning with 1% potassium hydroxide for at least 48 hours. Glycerol alone was used for final storage. Using a Kaiser light box and a 5 megapixel Fuji digital camera, images of the cleansed specimens were captured.

The bones of the craniofacial region of four-week-old TG and WT animals (n=5) were characterised using high-resolution digital radiography using a Faxitron model MX-20 digital radiography machine (Faxitron Corporation, Lincolnshire, IL, USA) to monitor phenotypic changes. The radiographic densities of the craniofacial region of the samples exposed simultaneously on the digital sensor and compared between the WT and the TG animals.

DMP1 is a physiologically active molecule that has been linked to a regulatory role during the early differentiation of osteoblasts and odontoblasts as well as the construction of the calcified extracellular matrix using a variety of techniques, including the creation of a *DMP1*-null animal. During early development, inactivation of the *DMP1* gene did not result in gross morphological defects in bone or dentin, in part because *DMP1* has many similarities to other proteins. Both the first eight residues of the core protein of a bone and the first 10 amino acid residues of the bone glycoprotein BAG-75's N-terminal sequence are identical.

Our investigation of the bone marrow stem cells further shows that the commitment and quick differentiation of these cells may be the cause of the mineralized phenotype to the lineage of osteoblasts. As a result, *DMP1*'s role as a positive regulator of the production of mineralized matrix is compatible with the heightened mineralization phenotype of the femurs at four weeks. This result is in line with our previously published research, which showed that overexpression of *DMP1* speeds up the maturation of preosteoblasts into fully differentiated osteoblasts and upregulates target genes that hasten osteoblast differentiation. Similar outcomes were attained when TG mice with ectopic Fra-1 expression improved their bone mass by controlling how osteoblasts and chondrocytes produce bone matrix.