Loss of fibulin-4 results in abnormal collagen fibril assembly in bone, caused by impaired lysyl oxidase processing and collagen cross-linking

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Abstract

The extracellular matrix protein fibulin-4 has been shown to be indispensable for elastic fiber assembly, but there is also evidence from human mutations that it is involved in controlling skeletal development and bone stability. Fibulin-4 mutations were identified in patients suffering from vascular abnormality and/or cutis laxa, and some of these patients exhibited bone fragility, arachnodactyly and joint laxity. In order to elucidate the role of fibulin-4 in bone structure and skeletal development, we analyzed structural changes in skeletal tissues of *Fbln4*$^{-/-}$ mice. Immunostaining confirmed that fibulin-4 is highly expressed in cartilage, bone, ligaments and tendons. No morphological abnormalities were found in the skeleton of *Fbln4*$^{-/-}$ mice as compared to wild type littermates except forelimb contractures as well as unusually thick collagen fibrils. Furthermore, fibulin-4 deficiency caused enhanced susceptibility of bone collagen for acid extraction, consistent with significantly reduced lysylpyridinoline and hydroxylysylpyridinoline cross-links in bone. In accordance with that, the amount of lysyl oxidase in long bones and calvaria was strongly decreased and proteolytic activation of lysyl oxidase was reduced in fibulin-4 deficient osteoblasts, while addition of recombinant fibulin-4 rescued the activation. The finding suggested that fibulin-4 is important for the proteolytic activation of lysyl oxidase which has a pivotal role in cross-linking of collagen and elastin.

Introduction

Collagens and elastin are major extracellular matrix proteins which serve important structural functions in the animal body. Type I collagen is the most abundant protein in vertebrates and comprises 90% of the organic components of bone. Intermolecular cross-links are essential for the functions of collagen and elastic fibers, and lysyl oxidase (LOX) is the responsible enzyme which initiates the first step of cross-link formation. Lysine (Lys) ε-amino groups in elastin are oxidatively deaminated by LOX to form allysine, which subsequently condense to yield desmosine or isodesmosine [1]. In collagen I, specific Lys and hydroxylysine (Hyl) residues in the N- and C-telopeptides are oxidatively deaminated to

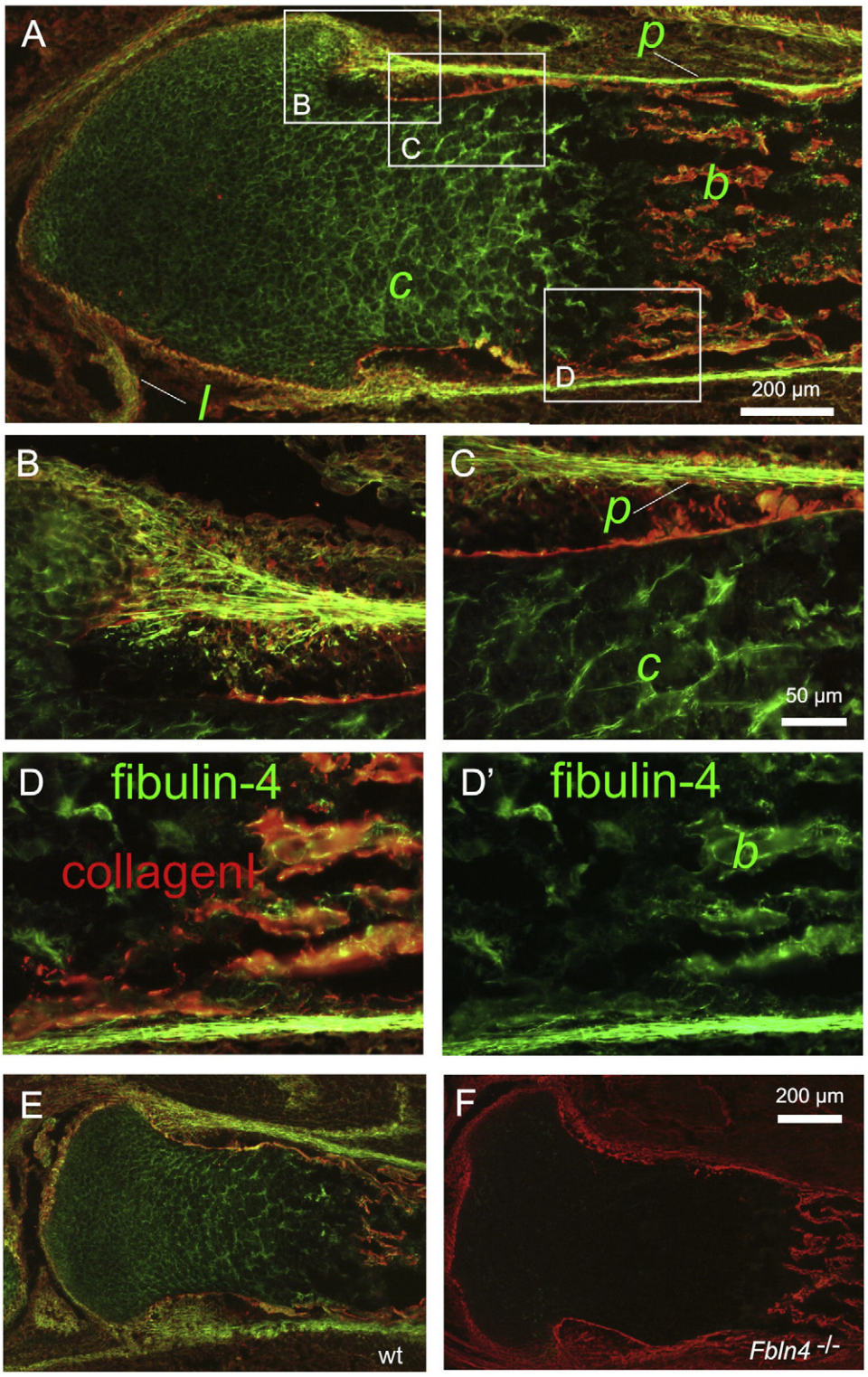

Fig. 1. Expression of fibulin-4 in mouse skeletal tissue. (A–D) Staining of the tibia and adjacent tissues from newborn mice with anti-fibulin-4 antibody (green) and anti-collagen I antibody (red) shows fibulin-4 in cartilage (*c*), ligaments (*l*), periosteum (*p*) and bone (*b*). (B–D, D') B–D labeled in A are shown with a higher magnification. D' is the same as D but only fibulin-4 staining is shown. (E, F) Staining of wild type (E) and *Fbln4*$^{-/-}$ femur (F) with the same antibodies as in A shows the absence of fibulin-4 in *Fbln4*$^{-/-}$ mice.