The ameloblastin extracellular matrix molecule enhances bone fracture resistance and promotes rapid bone fracture healing

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Abstract

The extracellular matrix (ECM) provides structural support, cell migration anchorage, cell differentiation cues, and fine-tuned cell proliferation signals during all stages of bone fracture healing, including cartilaginous callus formation, callus remodeling, and bony bridging of the fracture gap. In the present study we have defined the role of the extracellular matrix protein ameloblastin (AMBN) in fracture resistance and fracture healing of mouse long bones. To this end, long bones from WT and AMBN$^{\Delta 5-6}$ truncation model mice were subjected to biomechanical analysis, fracture healing assays, and stem cell colony formation comparisons. The effect of exogenous AMBN addition to fracture sites was also determined. Our data indicate that lack of a functional AMBN in the bone matrix resulted in 31% decreased femur bone mass and 40% reduced energy to failure. On a cellular level, AMBN function inhibition diminished the proliferative capacity of fracture repair callus cells, as evidenced by a 58% reduction in PCNA and a 40% reduction in Cyclin D1 gene expression, as well as PCNA immunohistochemistry. In terms of fracture healing, AMBN truncation was associated with an enhanced and prolonged chondrogenic phase, resulting in delayed mineralized tissue gene expression and delayed ossification of the fracture repair callus. Underscoring a role of AMBN in fracture healing, there was a 6.9-fold increase in AMBN expression at the fracture site one week after fracture, and distinct AMBN immunolabeling in the fracture gap. Finally, application of exogenous AMBN protein to bone fracture sites accelerated callus formation and bone fracture healing (33% increase in bone volume and 19% increase in bone mineral density), validating the findings of our AMBN loss of function studies. Together, these data demonstrate the functional importance of the AMBN extracellular matrix protein in bone fracture prevention and rapid fracture healing.

Introduction

The extracellular matrix (ECM) of bone is a dynamic meshwork of self-assembled macromolecules that exerts profound control over all aspects of bone cell fate and behavior, including cell proliferation, survival, shape, migration and differentiation [1–4]. The principal component of the bone ECM is type I collagen; however, the bone matrix is also rich in other non-collagenous structural proteins (e.g. fibronectin, laminin, and elastin), small integrin-binding proteins (SIBLING proteins, bone sialoprotein BSP, dentin matrix protein DMP1, matrix extracellular phosphoglycoprotein MEPE, and osteopontin OPN), matricellular proteins (e.g. tenascin-C, thrombospondin, SPARC), proteoglycans (asporin,

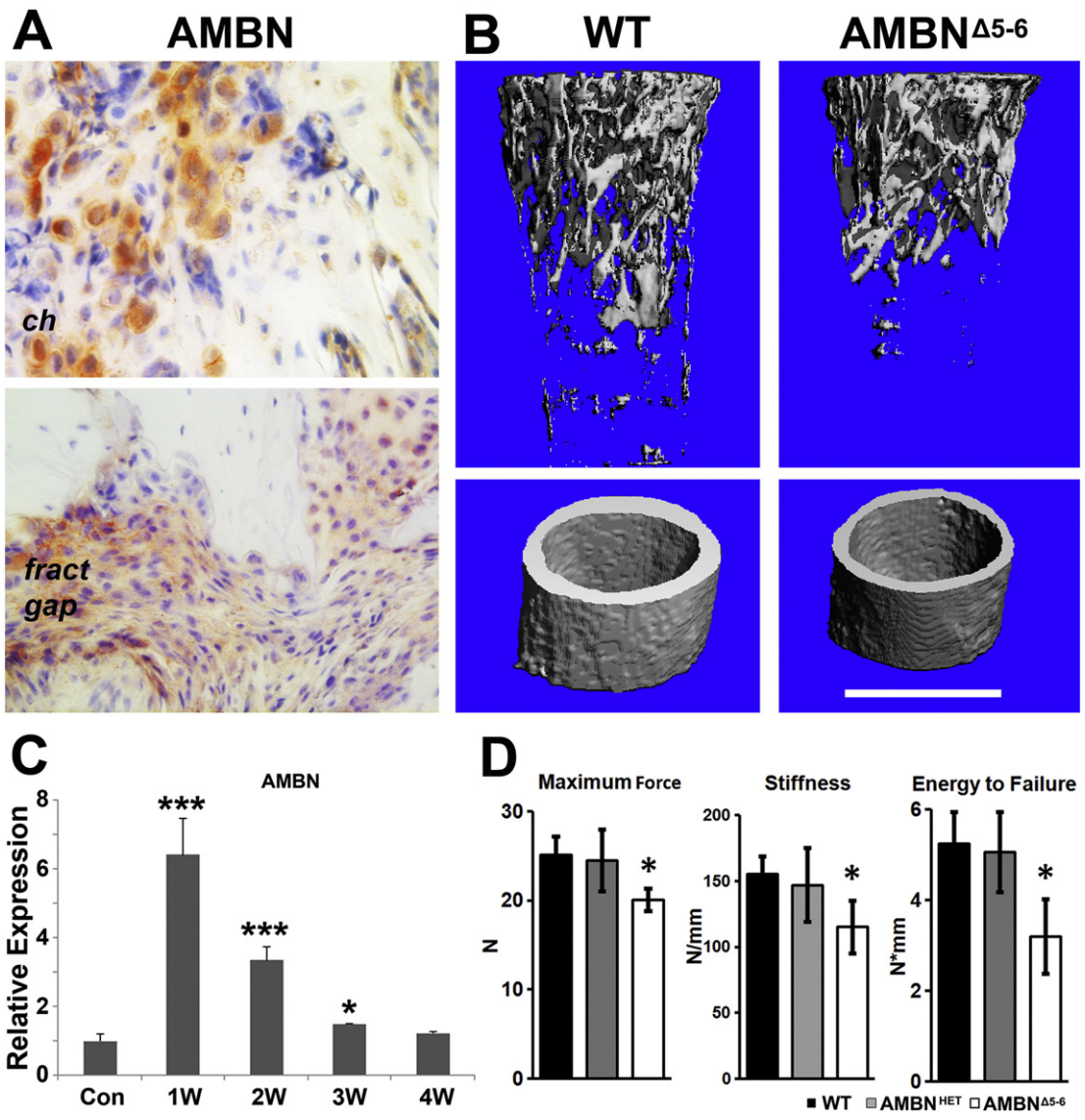

Fig. 1. Differences in bone structure and mechanical properties in AMBN mutant mice versus controls. (A) Immunohistochemical analysis of AMBN protein expression and localization in periosteal callus of WT mouse fractured tibia. (B) 3D reconstruction of μ-CT images from WT and AMBN$^{\Delta 5-6}$ femurs of 3 month old mice. Bar = 1 mm. (C) qRT-PCR analysis of Ambn gene expression during tibia fracture healing. (D) Maximum force, stiffness, and energy to failure measurements generated by femur 3-point bending tests. Data are presented as mean ± SD. *$p < 0.05$.

biglycan, decorin, keratocan), enzymes (e.g. metalloproteinases), and growth factors (e.g. insulin growth factors IGF, transforming growth factors TGF, and bone morphogenetic proteins BMP). This complex macromolecular network not only provides strength and connectivity to the mineralized apatite nanoskeleton of bone, but also allows for a dynamic and highly specific response to traumatic stimuli, including injury and fracture.

Ameloblastin (AMBN) is one such extracellular matrix protein that contributes to the many functions of the mineralized tissue matrix [5]. Originally discovered as the second most prominent enamel matrix protein [6,7], AMBN has since been detected in many other tissues including dentin, cementum, pulp, and cranial bones [8]. Explaining its multiple functions in development and regeneration, AMBN is related to the osteonectin (SPARC) ancestor SPARCL1, together with other enamel proteins including amelogenin and enamelin [9]. As a bone extracellular matrix protein, AMBN affects Msx signaling [10,11], integrin signaling [12], and cell adhesion through RhoA signaling [13].

The protein phase of the bone extracellular matrix plays important roles not only in bone development and maintenance, but also in response to bone injury