Matrix Biology 28 (2009) 292–303

Contents lists available at ScienceDirect

Matrix Biology

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

A mouse model expressing a truncated form of ameloblastin exhibits dental and junctional epithelium defects

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ARTICLE INFO

Article history:
Received 26 January 2009
Received in revised form 6 April 2009
Accepted 7 April 2009

Keywords:
Ameloblastin
Enamel organ
Junctional epithelium
Animal model
Mineralization

ABSTRACT

Ameloblastin (AMBN) is the second most abundant extracellular matrix protein produced by the epithelial cells called ameloblasts and is found mainly in forming dental enamel. Inactivation of its expression by gene knockout results in absence of the enamel layer and its replacement by a thin layer of dysplastic mineralized matrix. The objective of this study was to further characterize the enamel organ and mineralized matrix produced in the AMBN knockout mouse. However, in the course of our study, we unexpectedly found that this mouse is in fact a mutant that does not express the full-length protein but that produces a truncated form of AMBN. Mandibles from wild type and mutant mice were processed for morphological analyses and immunolabeling. Microdissected enamel organs and associated matrix were also prepared for molecular and biochemical analyses. In incisors from mutants, ameloblasts lost their polarized organization and the enamel organ detached from the tooth surface and became disorganized. A thin layer of dysplastic mineralized material was deposited onto dentin, and mineralized masses were present within the enamel organ. These mineralized materials generated lower backscattered electron contrast than normal enamel, and immunocytochemistry with colloidal gold revealed the presence of amelogenin, bone sialoprotein and osteopontin. In addition, the height of the alveolar bone was reduced, and the junctional epithelium lost its integrity. Immunochemical and RT–PCR results revealed that the altered enamel organ in the mutant mice produced a shorter AMBN protein that is translated from truncated RNA missing exons 5 and 6. These results indicate that absence of full-length protein and/or expression of an incomplete protein have direct/indirect effects beyond structuring of mineral during enamel formation, and highlight potential functional regions on the AMBN molecule.

1. Introduction

Ameloblastin (AMBN) is a member of the secretory calcium-binding phosphoprotein (SCPP) gene cluster of evolutionally-related molecules that regulate skeletal mineralization (Kawasaki and Weiss, 2003). It is the second most abundant matrix protein produced by ameloblasts and is generally believed to be located exclusively in forming enamel (reviewed in Hu et al., 2005). However, transient expression of AMBN was also found in differentiating odontoblasts (Bègue-Kirn et al., 1998; Hao et al., 2005; Simmons et al., 1998), during tooth root formation (Fong et al., 1996) and craniofacial bone development (Spahr et al., 2006). AMBN is short-lived and rapidly undergoes major C-terminal processing (Murakami et al., 1997; Uchida et al., 1997). The cleaved fragments leave the enamel layer while the N-terminal portions persist for longer periods (Nanci et al., 1998; Uchida et al., 1997). This unique protein contains potential sites for cell adhesion and for posttranslational modifications such as O-linked glycosylation, sulfation and phosphorylation (Cerny et al., 1996; Krebsbach et al., 1996). The phosphorylation modification includes a site for casein kinase II that is shared by other proteins involved in mineralization such as bone sialoprotein (BSP) and osteopontin (OPN) (Krebsbach et al., 1996). Newly secreted AMBN temporarily accumulates at enamel growth sites where crystals actively elongate (Nanci et al., 1998; Uchida et al., 1997). This has led to the suggestion that it may regulate crystal elongation (Hu et al., 2005;

Abbreviations: AMBN, ameloblastin; AMBN$^{\Delta 5-6}$, AMBN mouse lacking exons 5 and 6; AMBN^{HET}, AMBN heterozygote mouse; AMBN^{WT}, AMBN wild type mouse; AMEL, amelogenin; AMLN, amelin; BEI, backscattered electron imaging; BSP, bone sialoprotein; CT, connective tissue; dcw, distal cell web; dpTP, distal portions of Tomes' processes; EO, enamel organ; ES, enamel space; F, forward primer (5'-side region of DNA); HD, hemidesmosome; JE, junctional epithelium; M, maturation stage of amelogenesis; MA, apical maturation; MI, incisal maturation; N, nucleus; OPN, osteopontin; ORF, open reading frame; pA signal, polyadenylation signal; PDL, periodontal ligament; ppTP, proximal portion of Tomes' process; PS, presecretory stage of amelogenesis; R, reverse primer (3'-side of DNA); RT–PCR, reverse transcriptase polymerase chain reaction; S, secretory stage of amelogenesis; SCPP, secretory calcium-binding phosphoprotein; SDS–PAGE, SDS–polyacrylamide gel electrophoresis; SEM, scanning electron microscope; sg, secretory granules; TF, tonofilament; TSS, transcription start signal.

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doi:10.1016/j.matbio.2009.04.004

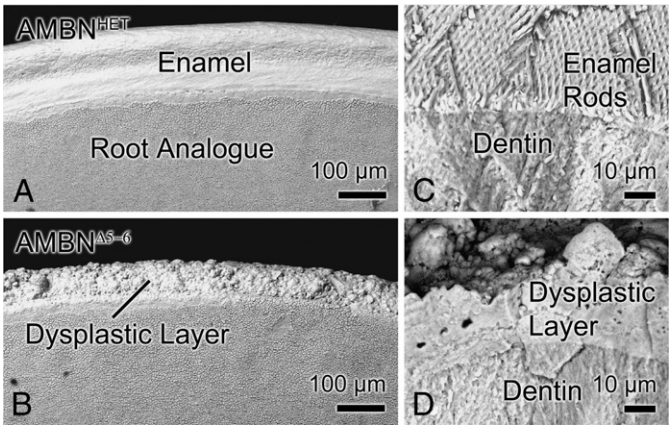

Fig. 1. SEM images of maxillary incisors from AMBN^{HET} (A, C) and AMBN$^{Δ5-6}$ (B, D) mice. (A, B) Surface and (C, D) fractured views. The enamel layer in AMBN^{HET} mice exhibits (A) a smooth surface and (C) a complex rod and interrod pattern. (B, D) Incisors from AMBN$^{Δ5-6}$ animals instead show a dysplastic layer composed of irregular mineralized masses with no defined structural pattern.

Nanci et al., 1998) perhaps as a result of its calcium-binding properties (Vymetal et al., 2008). For reasons that are still unknown, AMBN continues to be expressed throughout the maturation stage, long after the entire thickness of the enamel layer has been deposited (Lee et al., 2003; Nanci et al., 1998).

There are presently two animal models involving genetic manipulation of AMBN, a knockout (KO) mouse (Fukumoto et al., 2004) and a transgenic mouse overexpressing AMBN (Paine et al., 2003). In the AMBN KO mouse model, ameloblasts (and associated cells layers of the enamel organ) detach from the tooth surface as they enter the secretory stage. This directly or indirectly causes them to stop their typical differentiation sequence and abort enamel formation (Fukumoto et al., 2004). Instead, only a very thin layer of dysplastic mineralized material covers the dentin surface; the origin and nature of this material are presently unknown. Interestingly, overproduction of AMBN in transgenic mice leads to formation of thinner and more porous enamel, with disrupted rod patterns and abnormal crystallites (Paine et al., 2003). Together, these findings suggest that AMBN acts, in part, as a promoter of the process of enamel formation, but that it may also act as an inhibitor of enamel formation when present in quantities greater than its normal basal levels.

To broaden our understanding of the role of AMBN, we have therefore further examined the structure of the enamel organ and characterized the nature of the dysplastic mineralized matrix produced in the KO mouse (Fukumoto et al., 2004). Since the enamel organ is implicated in formation of the junctional epithelium (reviewed by Bosshardt and Lang, 2005; Shimono et al., 2003), we have also investigated whether detachment and disorganization of the enamel organ in this mouse model has consequences for development of a normal junctional epithelium when the tooth erupts. While the strategy used to create the AMBN KO mouse model was initially believed to fully abrogate gene and protein expression, we have now found that a truncated mRNA is still produced from the targeted allele. We therefore also carried out immunolabeling, biochemical and molecular analyses to characterize the expressed protein. Our results show that AMBN influences both cells and matrix events and that absence of the full-length protein and/or expression of an incomplete protein has effects beyond the structuring of mineral during enamel formation. Identification of the portion of AMBN protein still produced in this mouse model provides new insights into the functional domains that may be implicated in these activities.

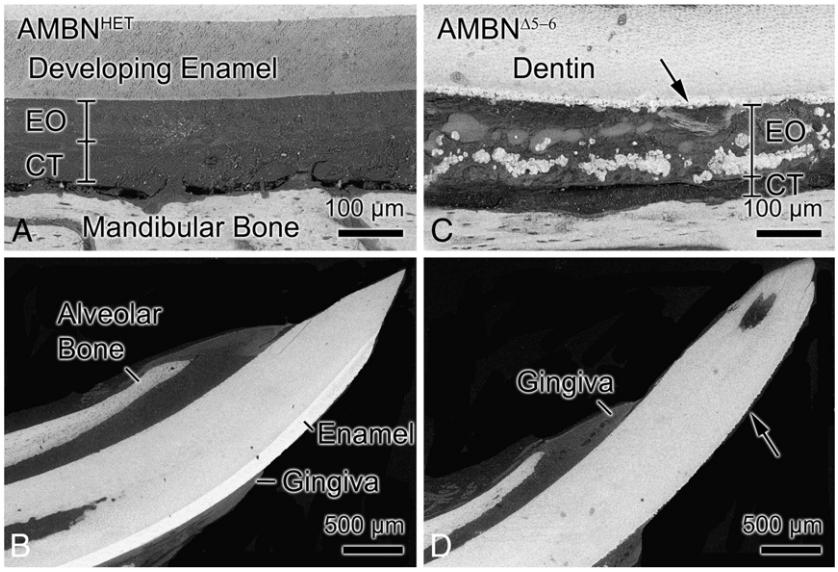

Fig. 2. Backscatter SEM images of sagittally cut incisors from AMBN^{HET} (A, B) and AMBN$^{Δ5-6}$ (C, D) mice from corresponding late secretory (A, C) and erupted (B, D) portions of the tooth. The absence of full-length AMBN (B) results in lack of formation of an enamel layer and its replacement by a thin, dysplastic mineralized layer along the dentin surface (arrow), and the presence of mineralized masses throughout the disorganized enamel organ (EO). (D) A shift of the gingival margin with respect to the tip of the incisor and the alveolar bone is also observed. CT, connective tissue.