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Review

TGF-β autocrine signaling at secretory-stage enamel

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ABSTRACT

Background: In recent years, transforming growth factor (TGF)-β has been found in the enamel matrix, and along with enamel protein, is thought to play an important role in the process of calcification of tooth enamel. The purpose of this study was to investigate the dynamics of TGF-β and its interactions with enamel proteins, through experiments at the genetic and protein levels.
Highlights: The expression of the latent *TGF-β1* gene was observed during the enamel formation process. TGF-β1 was found in both immature and mature enamel, and its activity tended to decrease as immature enamel transitioned to mature enamel. *In vitro* studies showed that latent TGF-β1 was activated by matrix metalloproteinase 20 (MMP20), and activated TGF-β1 was degraded by kallikrein-4 (KLK4). By binding to the major amelogenins, activated TGF-β1 maintained its activity. Of the major amelogenins, the 13 kDa amelogenin had the highest affinity for activated TGF-β1. Moreover, the 13 kDa amelogenin-activated TGF-β1 complex was bound to the TGF-β1 receptor on the ameloblast cell surface and induced signaling.
Conclusion: Latent TGF-β1 produced and secreted from secretory-stage ameloblasts is activated by MMP20, and the activated TGF-β1 maintains its activity by combining with amelogenin cleavage products processed by the same protease. TGF-β1 moves through the aqueous phase with the water-soluble 13 kDa amelogenin and binds to its receptor on the ameloblast surface, thereby inducing autocrine signaling. Once the ameloblasts differentiate and enter the maturation stage, TGF-β1 is degraded by KLK4, which is produced and secreted by maturation-stage ameloblasts, and loses its activity.

Contents

Abbreviations: ALP, alkaline phosphatase; BMP, bone morphogenic protein; ELISA, enzyme-linked immunosorbent assay; HPLC, high performance liquid chromatography; MMP, matrix metalloproteinase; qPCR, quantitative polymerase chain reaction; RT-PCR, reverse transcription-polymerase chain reaction; SDS-PAGE, sodium dodecyl sulfate-polyacrylamide gel electrophoresis; TGF-β, transforming growth factor-beta; TGFBR1, transforming growth factor-beta receptor I

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1. Introduction

Tooth enamel is highly calcified and is the hardest tissue in the human body. Its formation begins when ameloblasts form proteinaceous material. The process of enamel formation occurs in three stages, namely, the, secretory, transition, and maturation stages, and in the end enamel is composed primarily of minerals. During the secretory stage, minerals accumulate in a long, thin, ribbon-like manner along the calcification front on the enamel surface. The ribbon-like minerals slowly transition to hydroxyapatite crystals, and after the enamel reaches its final thickness during the maturation stage, maturation of the crystals occurs. In this microenvironment, enamel proteins around the crystals are intimately involved in their temporal and spatial development. The enamel proteins in the secretory-stage (immature) enamel include amelogenin, ameloblastin, and enamelin, in descending order of abundance. These enamel proteins are produced and secreted by ameloblasts, and are slowly processed by matrix metalloproteinase 20 (MMP20) [1–3]. These proteins are later degraded by kallikrein 4 (KLK4), which is secreted from ameloblasts in the secretory and maturation stages [4].

In addition to the abovementioned enamel proteins and proteases, TGF-β isoforms secreted from ameloblasts have been reported to be present in the enamel matrix [5]. TGF-β1 regulates mRNA expression of both *MMP20* and *KLK4* [6,7]. Moreover, through the secretion of KLK4, TGF-β1 regulates the calcification and maturation of enamel [8], and its expression in the maturation-stage enamel organ increases, which may induce apoptosis in ameloblasts [9]. Other studies have reported that SMAD3 is necessary for enamel calcification, and that TGF-β is important for its signaling [10]. Overexpression of TGF-β1 in secretory-stage ameloblasts leads to an abnormal enamel calcification pattern [11]. Many investigators have focused on the fact that problems with interactions not only at the genetic level but also at the protein level result in abnormal enamel formation. However, studies related to TGF-β in enamel formation as described above have been limited mostly to the genetic level, and much remains unknown about the dynamics and roles of TGF-β. We previously reported that TGF-β exists in the porcine secretory-stage and maturation-stage enamel matrix, and that TGF-β increases the activity of alkaline phosphatase (ALP) in human periodontal ligament (PDL) cells [12].

In this review, we describe previously obtained results regarding the mechanism of normal enamel formation, focusing particularly on TGF-β1, and discuss the activation and inactivation mechanisms, protein–protein interactions, and TGF-β-induced signaling, to elucidate the dynamics at the protein level, as well as the genetic level. We also report that in enamel formation, TGF-β1 influences various enamel proteins in a complicated autocrine system.

2. Gene expression and activation of TGF-β1 in the enamel formation process

TGF-β is produced and secreted by cells throughout the body and affects various physiologic processes. However, the question of how it is expressed and activated within the enamel matrix during the enamel formation process has yet to be clarified. We used a 5-month-old pig model to first investigate *TGF-β* gene expression. In preparing total RNA, we took care in determining which of the three stages of the formation process the enamel was in. We previously reported that when extracting a tooth from tooth germ, the enamel organ epithelium (EOE) corresponding to the secretory stage can be removed easily from the tooth, but the EOE corresponding to the maturation stage remains adhered to the tooth [13]. Accordingly, we prepared total RNA from secretory- and maturation-stage EOE. Moreover, secretory- and maturation-stage enamel have a cheese-like (solid but not hard) and chalk-like consistency, respectively. Thus, EOE in the border of these regions was considered to be in a transition stage, and total RNA from this region was prepared (Fig. 1A).

MMP20 is expressed by secretory-stage ameloblasts [1–3], whereas KLK4 is specifically expressed by transition- and maturation-stage ameloblasts [2,14]. In the quantitative PCR (qPCR) analysis based on our sample preparation described above, the mRNA level of *Mmp20* was clearly high in porcine secretory-stage EOE, while that of *Klk4* was clearly high in porcine transition-stage

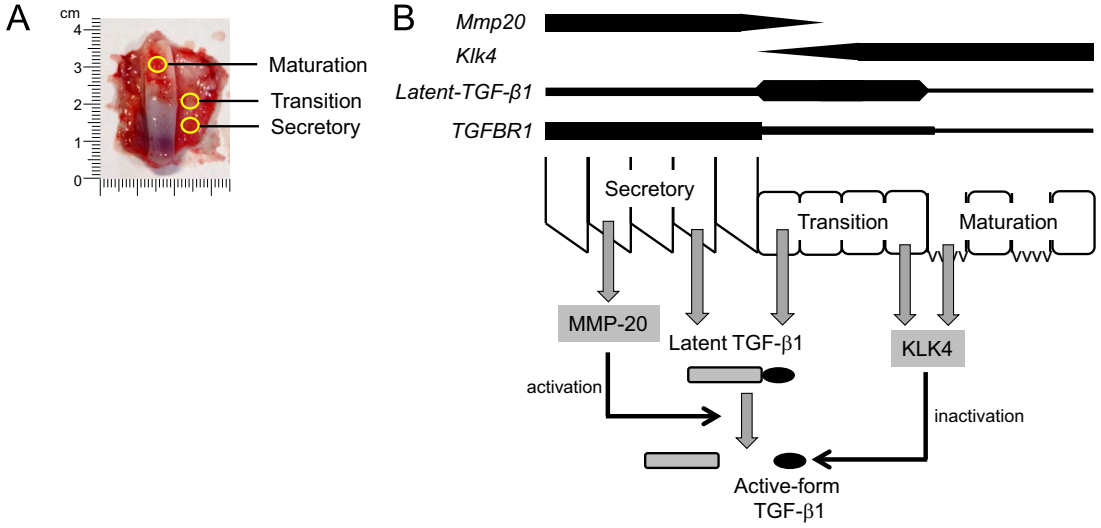

Fig. 1. Activation and inactivation of TGF-β during enamel formation. ***A***: Permanent incisor and enamel organ epithelia from a 5-month-old pig used for genetic study. The secretory-, transition- and maturation-stage ameloblast layers were excised with a razor blade. On the permanent incisor of a 5-month-old pig, the secretory ameloblasts are separated from the enamel layer along with the rest of the enamel organ epithelia. In contrast, the maturation-stage ameloblasts are adherent to the underlying enamel layer because the basement membrane of maturation-stage ameloblasts mediate the attachment of those epithelial cells to the mineralized tooth surface. ***B***: Schema of expression of *Mmp20*, *Klk4*, *latent TGF-β1*, and *TGFBR1* genes during enamel formation, and activation and inactivation of the latent TGF-β1 by MMP20 and KLK4.

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