A Potential Mechanism for the Development of Dental Fluorosis

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Abstract. Currently, the mechanism behind the development of dental fluorosis remains unclear, but it is known that fluorotic enamel has higher protein content and is therefore softer than nonfluorosed enamel. Previously it was demonstrated that fluoride induces phosphorylation of the eIF2α ribosomal component, which significantly decreases protein synthesis. This occurs during the maturation stage of development when proteins are normally removed from the hardening enamel. By combining these data with current knowledge of ameloblast function during enamel development, we can hypothesize a potential mechanism in which excess fluoride results in increased protein levels and softened enamel via decreased protease secretion during the maturation stage. Briefly, this hypothesis states that phospho-eIF2α-mediated inhibition of protein production induced by intracellular fluoride results in decreased secretion of the enamel protease kallikrein-4 (KLK4) during the enamel maturation phase. This in turn results in decreased protein breakdown and higher protein content within the enamel matrix.

Key words. Ameloblast, Dental fluorosis, eIF2α, Enamel, Fluoride

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1 Dental Fluorosis

Fluoride treatment, via either ingestion or topical methods, has been shown to significantly reduce the prevalence of dental caries in both developing and developed teeth. Incorporation of fluoride ions into the hydroxyapatite crystals of enamel increases hardness and reduces demineralization, which can delay caries formation. Excess fluoride, however, has a detrimental effect on developing enamel, resulting in opaque mottling, pitting and, in severe cases, discoloration of enamel. These areas of fluorotic enamel are undermineralized, containing increased levels of protein when compared to areas of nonfluorosed enamel [1–3]. The mechanism responsible for developing fluorotic enamel remains poorly understood.

2 Enamel Development

Enamel is produced by specialized epithelial cells called ameloblasts, which are polarized columnar cells capable of secreting large quantities of proteins. The chief function of an ameloblast is to support the growth of a single hydroxyapatite enamel rod by secreting scaffold proteins during the secretory stage, in which the rods growth in length, and then removing these proteins during the maturation stage, in which the rods thicken. There are several stages to the life cycle of an ameloblast, the early phase, called the presecretory stage, focuses on cell growth and differentiation, ensuring the cellular machinery, such as the endoplasmic reticulum, are sufficient for the next stage. In the secretory stage, ameloblasts secrete enamel scaffold proteins, which support the growing enamel crystals, and the protease matrix metalloproteinase-20 (MMP20, enamelysin), which cleaves these scaffold proteins, a step required for their proper function (reviewed in [4]). At this stage, the developing enamel is extremely soft, containing a high amount of protein, and the decussating enamel rod pattern begins to form. Additionally, in this stage, the apical end of the ameloblast forms the Tomes' process, a conical-shaped extension of the cell surface from which proteins are secreted into the enamel matrix. Ameloblasts transition into the maturation phase by shortening, retracting their Tomes' processes, and gaining either a ruffle—or smooth-ended apical cell surface. During this stage, MMP20 secretion decreases and secretion of kallikrein-4 (KLK4) increases. Like MMP20, KLK4 also cleaves the scaffold proteins in the enamel matrix but instead of being required for proper function, this facilitates their resorption by the ameloblasts. This ultimately results in a reduction of the enamel protein content from greater than 30% to less than 1% [5] and allows the enamel rods further room for growth in both width and thickness. By the end of the maturation stage, the enamel is fully hardened. In summary: ameloblasts secrete stage-specific proteases crucial for enamel rod growth and maturation as well as absorbing digested protein fragments necessary for increasing enamel hardness.

3 The Acid Hypothesis

As previously mentioned, the exact mechanism behind the development of dental fluorosis is currently unknown. However, fluoride has been shown to have no effect on the specific activity of either MMP20 or KLK4, ruling out inactivation of the enamel proteases during development as a possible mechanism [6]. During the secretory stage, the neutral pH of the enamel matrix remains stable, but in the maturation stage the pH oscillates from neutral to mildly acidic (reviewed in [7]). This results from deposition of hydroxyapatite, which releases hydrogen ions during crystallization and, in turn, decreases the pH of the enamel matrix. In addition to their secretion and absorption functions during enamel maturation mentioned above, ameloblasts also control the pH of the enamel matrix by secreting bicarbonate into the matrix and utilizing ion transporters to absorb hydrogen ions from the matrix. Previously our group has hypothesized that the presence of fluoride during the acidic phases of enamel maturation results in the formation of increased levels of highly toxic hydrogen fluoride (HF) [8]. Because HF is a weak acid and can easily defuse through cell membranes, it diffuses down a concentration gradient into the cell. Once in the neutral cytosol of the cell, hydrogen fluoride would then dissociate into its component ions. The Henderson–Hasselbalch equation states that 25-fold more HF is present at a pH of 6.0 than at a pH of 7.4. It was demonstrated that activation of stress response genes occurred at lower fluoride doses and that fluoride-mediated inhibition of protein secretion was increased under acidic conditions when compared to neutral conditions [8]. Sharma et al. [8] hypothesized this was a result of increased intracellular fluoride levels in cells grown under acidic conditions.

4 Intracellular Stress Responses to Fluoride

Previously our group has shown that inactivation of the translational control protein eukaryotic initiation factor 2 (eIF2) via phosphorylation on its alpha subunit (eIF2α) occurs in both cultured cells and in ameloblasts from mice exposed to fluoride [8–10]. The unfolded protein response (UPR) responds to misfolded proteins in the ER by increasing transcription of chaperones and stress response genes, and also by generally decreasing overall protein synthesis, which in turn decreases ER load. During the UPR, the PERK kinase acts to decrease protein synthesis via an inhibitory phosphorylation of eIF2α at S51 [11]. This prevents eIF2B from hydrolyzing GTP and initiating the elongation step of protein translation. eIF2α can also be phosphorylated by three other known kinases, each responding to different stimuli: heme-regulated inhibitor (HRI) responds to heme deprivation, arsenite exposure, heat shock and oxidative stress; protein kinase RNA-activated (PKR) in response to dsRNA resulting from viral infection; and general control nonrepressed 2 (GCN2) in response to UV exposure and nutrient limitation (reviewed in [12]). Other groups have shown activation of PERK-mediated signaling in response to fluoride in cultured osteoblasts suggesting PERK plays an important role during the development of

skeletal fluorosis [13]. 3-D culture of these cells in the presence of fluoride also resulted in increased levels of reactive oxygen species, which induced oxidative response pathways [14], and HRI has been shown to be activated by Hsp90 in response to oxidative stress [15, 16]. Whether fluoride activates PERK via ER-stress or HRI via oxidative stress or both, these data provide a strong foundation to begin investigations into the role of the eIF2α kinases during the cellular response to fluoride.

5 Combined Theory of Dental Fluorosis

The Acid Hypothesis, described above, states that, due to pH fluctuations in the enamel matrix during the maturation phase, HF is able to diffuse down a concentration gradient into the cytosol where it dissociates into its component ions [8]. In vivo and in vitro data show fluoride exposure results in eIF2α phosphorylation [8, 10], which is a well-characterized mechanism for halting protein synthesis [12]. qPCR on enamel organs from mice exposed to fluoride also show decreased levels of maturation stage-specific mRNA, KLK4 and amelotin (Amtn) [8]. By combining these data, we can conclude that excess intracellular fluoride results in decreased KLK4 production and secretion via phospho-eIF2α, which accounts for the increased levels of protein seen in fluorotic enamel. Ongoing work is focused on determining which of the upstream eIF2α kinases is involved in the intracellular response to fluoride during the acidic maturation phase as well as determining the extent, if any, of the involvement of UPR stress pathway.

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