Review

Keratins as components of the enamel organic matrix

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Abstract

Dental enamel is the hardest tissue in the human body, and although it starts as a tissue rich in proteins, by the time of eruption of the tooth in the oral cavity only a small fraction of the protein remains. While this organic matrix of enamel represents less than 1% by weight it plays essential roles in improving both toughness and resilience to chemical attacks. Despite the fact that the first studies of the enamel matrix began in the 19th century, its exact composition and mechanisms of its function remain poorly understood. It was proposed that keratin or a keratin-like primitive epithelial component exists in mature enamel, however due to the extreme insolubility of its organic matrix the presence of keratins there was never clearly established. We have recently identified expression of a number of hair keratins in ameloblasts, the enamel secreting cells, and demonstrated their incorporation into mature enamel. Mutation in epithelial hair keratin *KRT75* leads to a skin condition called pseudofollicularis barbae. Carriers of this mutation have an altered enamel structure and mechanical properties. Importantly, these individuals have a much higher prevalence of caries. To the best of our knowledge, this is the first study showing a direct link between a mutation in a protein-coding region of a gene and increased caries rates. In this paper we present an overview of the evidence of keratin-like material in enamel that has accumulated over the last 150 years. Furthermore, we propose potential mechanisms of action of KTR75 in enamel and highlight the clinical implications of the link between mutations in KRT75 and caries. Finally, we discuss the potential use of keratins for enamel repair.

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Enamel: a brief overview

Dental enamel comprises the outer layer of a tooth crown and is the hardest tissue of the human body. It is composed of ~96% carbonated apatite, ~3% of water and less than 1% of organic matrix by weight. Although the organic matrix is a minor component of mature enamel, it plays a very important role in the mechanical toughening of this tissue [1–3]. The basic building block of enamel is the enamel rod, which consists of elongated crystals, arranged in parallel arrays with their crystallographic c-axes perfectly co-aligned (Fig. 1A). Enamel rods are approximately 2–3 μm in diameter and are wrapped in a thin layer of organic matrix called enamel rod sheaths. Even though the organic matrix is present throughout the enamel thickness, its concentration is greater in the inner enamel layer where, in addition to the rod sheaths, larger organic structures called enamel tufts are present at the interface with dentin [4].

Ameloblasts are epithelial cells responsible for enamel deposition. They start to secrete a mineralized extracellular matrix on top of the dentin soon after the onset of dentin mineralization, and this stage of enamel deposition is called secretory stage. The composition of secretory enamel is very different from that of mature enamel; it consists of roughly equal parts of mineral, organics and water by weight.

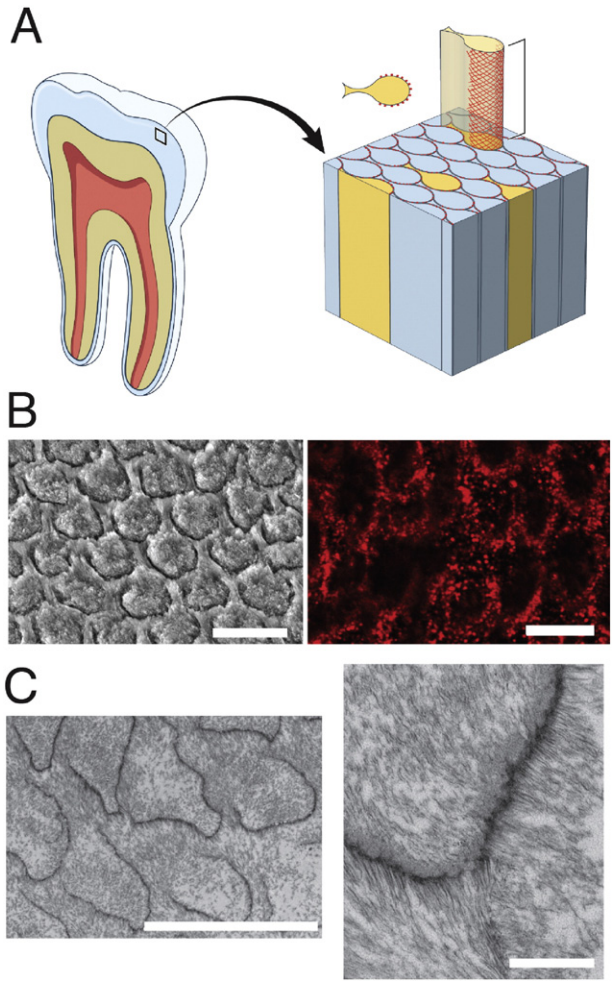

Fig. 1. Enamel structure and the presence of hair keratins in enamel rod sheaths. A) Schematics showing the arrangement of enamel rods in mature enamel and their association with enamel rod sheaths made of organic material accumulated along a semi-circle at the periphery of each rod. B) Scanning electron microscopy analysis of ground, polished and etched human molars showing the characteristic keyhole pattern of enamel rods (left panel; scale bar: 10 μm). Immunochemical detection of KRT75 performed on a similar surface showing staining primarily where enamel rod sheaths are located (right panel; scale bar: 10 μm). Primary antibody: anti-KRT75 (LifeSpan BioSciences Inc.). Secondary antibody: Alexa 555 conjugated goat anti-guinea-pig antibody (Life technologies). C) Transmission electron microscopy of enamel rod sheaths after demineralization of human enamel showing the semi-circular pattern of sheaths surrounding each individual rod. Scale bars: left panel 10 μm; right panel 1 μm.

Importantly the structural organization of crystals in secretory and mature enamel is similar; the only difference is that the nascent crystallites are much thinner. The organic matrix of secretory enamel is composed primarily of a protein amelogenin, which accounts for 90% of the total protein [5,6]. Other matrix components include the structural proteins enamelin and ameloblastin, and a proteinase MMP20 [6]. When the full thickness of enamel is deposited, secretory ameloblasts transform into maturation stage ameloblasts. During the maturation stage, the enamel matrix proteins are degraded by proteinases such as KLK4 and replaced by fluid in which enamel crystals grow laterally, until the density of mature enamel is reached [6,7]. A very small organic fraction made of small peptides, amino acids and an insoluble proteinaceous material in the tufts and enamel rod sheaths remains in mature enamel.

A historical perspective

Early studies of mature enamel composition in the second part of the 19th century were focused on the question of enamel vitality, with some researchers proposing that enamel is a vital tissue due to its sensitivity to instruments, and others suggesting that enamel is a mineralized tissue lacking vital functions