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Possible roles of Sp6 in ameloblast differentiation

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Sp6/Epiprofin is a member of Sp/KLF family, which has the tandem three zinc finger domains. Compared to the other Sp/KLF family members, Sp6 has three unique features; no Sp domain, proline rich sequences at N-terminus, and tissue-specific expression (tooth, limb, and hair follicles). Sp6-deficient mice present the severe tooth defects together with the systemic defects, indicating that Sp6 may play a critical role in tooth development. However, the precise function of Sp6 remains unclear. We have found that a two-base insertion in the 3rd zinc finger domain of *Sp6* as causative gene mutation of a spontaneous amelogenesis imperfecta rat, AMI. Enamel is the hardest tissue of our body covering tooth surface and the enamel matrix is produced by ameloblasts derived from dental epithelial cells. To study the role of Sp6 in amelogenesis, we prepared the dental epithelial cell lines from AMI and control WT rat, named ARE-B30 and G5, respectively, and established the *in vitro* culture systems mimicking the *in vivo* cellular composition; the epithelial cells, G5 or ARE-B30, the matrix (collagen type I membrane), and the mesenchymal cells, RPC-C2A, a rat pulp cell line. By comparative analysis of gene expression, we found that the early and late stage-specific expression of ameloblast differentiation markers were detected in G5, but not in ARE-B30. To further assess the causative link between Sp6 and aberrant gene expression, we performed two functional analyses; 1) loss-of-function; Mithramycin A, GC-box inhibitor and siRNA were used in G5, and 2) gain-of-function; *Sp6* expression vector was introduced into ARE-B30 and G5 by transient transfection. The amelogenesis stage-specific gene expression was analyzed by semi-quantitative RT-PCR. Loss-of-function study showed the specific reduction of the amelogenesis-related gene expression, suggesting that Sp6 may have a role in these gene expression. However, gain-of-function study failed to restore the gene expression. To understand this discrepancy, currently we are analyzing the epigenetic regulation in this marker gene regulation.