O24-INDUCTION OF HUMAN KERATINOCYTES INTO ENAMEL -SECRETING AMELOBLASTS

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Introduction
Tooth crown forms through differentiation of dentin-secreting odontoblasts, and enamel producing ameloblasts. Utilization of stem cells from a patient to develop bioengineered replacement teeth is the ultimate goal of regenerative dental medicine. Various postnatal mesenchymal stem cells have been shown capable of differentiating into odontoblasts and creating mineralized dentin(*1-3*). However, identification of stem cells that can be used as the epithelial component for tooth regeneration remains a challenge. Cultured human skin keratinocytes have promised many prominent clinical applications. We tested if keratinocytes could represent an applicable source for enamel production.

Materials and Methods
Keratinocyte stem cells, isolated from circumcised human foreskins and confirmed by the expression of several molecular markers, were cultured to form confluent epithelial sheets. These epithelial sheets were recombined with E13.5 mouse molar mesenchyme that possesses an odontogenic potential(*4*). Recombinants with or without growth factors carried by agarose beads were subjected to subrenal culture.

Results
The recombinants developed into whole tooth crowns consisting of human and mouse tissues in 25% cases after 4-week culture under the mouse renal capsule. Immunohistochemical studies using specific antibodies against human or mouse MHC I antigens confirmed human origin of the epithelial component and mouse origin of the dental pulp, respectively. Histological examination revealed the presence of well-formed dentin, but a lack of enamel due to failed differentiation of the keratinocytederived epithelial cells into elongated ameloblasts. The human keratinocyte-derived epithelium thus supports mouse dental mesenchyme to form tooth structures but lacks ameloblastic differentiation capability under such condition. Next, we surveyed the potentials of several key growth factors to induce human keratinocytes into ameloblasts. Among them are SHH and BMP4 that are expressed in differentiating ameloblasts of mouse and/or human teeth(*5-6*), and FGF8 which is expressed in both odontoblasts and ameloblasts of developing human teeth. Growth factor-soaked beads were implanted in tissue recombinants of cultured human keratinocyte sheet and mouse molar mesenchyme, which were subsequently subjected to subrenal culture for 4-week. Neither SHH nor BMP4 induced ameloblast differentiation, while BMP4 rather caused bone formation instead of tooth in the recombinants. In contrast, inclusion of FGF8 beads, although did not enhance the success rate of tooth formation in the recombinants, indeed induced the elongation of keratinocyte-derived epithelial cells and the deposition of enamel in 6 cases of the 22 formed teeth. Immunostaining assays showed specific presence of ameloblastin and MMP-20, specific markers for differentiated ameloblasts, in the elongated epithelial cells and the enamel, confirming ameloblastic differentiation.

Discussion
In the presence of appropriate odontogenic signals, human keratinocytes can be induced to become odontogenic competent; and that these are capable of participating in tooth crown morphogenesis and differentiating into ameloblasts. It is interesting to note that in developing human embryo, the deciduous teeth begin to develop in the 6th week of gestation, but it is not until the 28th week the dental epithelium starts to differentiate into enamel-producing ameloblasts. However, in the human-mouse chimeric teeth, differentiated ameloblasts and deposited enamel are found within 4-week under the subrenal culture. This is likely due to a faster differentiation of the mouse odontoblasts, which in turn induces earlier ameloblastic differentiation of the human keratinocytes.

Conclusions
Our results demonstrate that human keratinocytes are capable of differentiating into ameloblasts and producing enamel, and identify the type of cell an appropriate source for generating bioengineered whole tooth crown for replacement therapy in the near future.

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