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Significance of Hertwig's Epithelial Root Sheath

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INTRODUCTION

As tooth crown formation completes and root formation begins, the inner and outer enamel epithelial cells proliferate from the cervical loop of the enamel organ to form a continuous sheath containing double layer of cells, which is known as Hertwig's epithelial root sheath (HERS). Through reciprocal epithelial-mesenchymal interactions, HERS plays an important role in root development, including root morphogenesis (root size, shape and number), dentinogenesis and cemetogenesis. However, the precise nature of this role remains unclear. At later stage of root development, following the formation of first radicular mantle dentin, HERS fragments into discrete clusters of cells and migrate away from the root to the region of future periodontal ligament, which form the epithelia cell rests of Malassez (ERM).

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Development of HERS

HERS consists of the inner and outer enamel epithelial cells excluding the stratum intermedium and stellate reticulum. The cells of the inner enamel epithelium (IEE) are square and the ones of the outer enamel epithelium (OEE) are rectangular[1, 2]. And the width of the OEE cells is higher than that of IEE cells throughout the period of root development[3]. In mouse injected with siRNA targeting ameloblastin (AMBN), HERS revealed a multilayered appearance and 5-bromo-2'-deoxyuridine

(BrdU) positive cells increased in the outer layers[4].

In some researches of mice and rat molars, during root formation, the number of HERS cells in normal molars reduces gradually, and total HERS length decreased after PN15, which may be associated with occlusion and sequentially effect on root elongation[3,5,6]. It was reported that the presence of insulin-like growth factor-I (IGF-I) resulted in elongation of HERS and increased cell proliferation in its outer layer in mouse molar[7]. Hepatocyte growth factor (Hgf), as one of the mediators of epithelial-mesenchymal interactions in rodent tooth, stimulates proliferation of HERS cells in culture and HERS elongation in an organ-culture system. This effect is downregulated when an antibody against the Hgf receptor is added to the culture medium[8]. In the contrary, excess epidermal growth factor (Egf) signaling caused a disordered rate of cell proliferation in IEE and OEE and inhibited HERS formation[9]. Additionally, BMP4 also inhibits HERS elongation under in vitro organ culture system[10].

Function of HERS

The classic theory on HERS function relates HERS with the establishment of root shape during root formation[1]. The proposed roles of HERS in root formation rang from structural division of dental ectomesenchymal tissues to dental follicle and dental papilla, regulating tooth-root shape and numbers, inducing the differentiation of mesenchymal stromal/stem cells into odontoblasts and cementoblasts, and acting as a precursor of cementoblast cell[11].

At the onset of HERS elongation from the cervical loop, HERS cells extend in apical direction, structurally separating dental follicle and dental pulp[12]. It is believed that HERS is the inducer and regulator of root formation determining the shape, size and number of roots. The closure patterning of the pulp chamber floor and the fusion of tooth roots are determined by the separating and elongating patterns of HERS[13,14]. HERS also plays an essential role in determining root number. At approximately postnatal day 12, the buccal and lingual processes of the first mouse molar-the multi-root tooth-grow horizontally and contact each other to form the furcation of the tooth and give rise to the root contour. After segregation into multi-root canals, each root continues its elongation to complete root formation[15,16].

Two types of epithelial-mesenchymal interactions occur during root formation, through which HERS functions as an inducer of odontogenesis and cementogenesis. One of these is the interaction occurring between HERS and the dental

papillae. It has been well accepted that the inner epithelial cells of HERS induces dental papilla cells to differentiate into odontoblasts[14,17]. TGF-β/BMP signaling plays an important role on this progress, that HERS secretes TGF-β, which induces the differentiation of dental papilla cells into odontoblasts, and BMP4 also function as a stimulator for odontoblast differentiation during root development[10,13,18]. Besides, dental papilla cells are stimulated by Sonic Hedgehog (Shh) and enamel matrix protein released from HERS, thereby compelling them to differentiate into root dentin formative odontoblasts[19,20]. Laminin 5 is also reported as secreted by HERS and can induce dental papilla cell attachment, growth, migration and differentiation[21].

The second epithelial-mesenchymal interaction occurs between HERS and the dental follicle - formation of periodontal tissues, including the cementum, periodontal ligament, and alveolar bone. According to the classic theory, HERS initiates cementoblast differentiation. As HERS starts disintegrating and form fenestrations, mesenchymal cells from the surrounding dental follicle enter through these fenestration and contact the newly formed dentin. These mesenchymal cells then differentiate to form cementoblasts forming cementum. It has also been proposed that HERS may be the progenitor cells for other mesenchymal cell populations within the periodontal ligament[22].

By far, the origin of cementoblast is controversial. Excluding that cementoblasts rise from mesenchymal cells of dental follicle, another possibility is that HERS cells undergo an epithelial-mesenchymal transformation and become functional cementoblasts. In a research of porcine root formation, the osteogenic capacity of HERS in vitro strongly supports the notion that HERS is capable of producing cementum directly. Electron microscopic and immunocytochemical analyses suggest that HERS is the cellular origin of the cementoblasts. Transmission electron microscopy data revealed that dissipated HERS cells acquire the typical morphological features of cementoblasts during the very early stage of porcine root formation, which can be explained by a phenotypic conversion of epithelial mesenchymal transition[23]. However, in the contrary, a recent study on rat molar cementogenesis, using keratin-vimentin and keratin-runt-related transcription factor 2 (Runx2) double immune-labelling, reported that HERS cells were unable to transform to an intermediate phenotype from epithelial to mesenchymal cells to give rise to cementoblasts[24].

Various genes, including TGF-β, BMPs, FGFs, Egfr, Shh, Notch, Gli, Msx2, HGF and others, are involved in HERS during the process of root development[25].

Previous studies have suggested that BMP4 and MSX2 are involved in epithelial-mesenchymal interactions during root development[26]. *Bmp-2* and *Bmp-4* were detected in both IEE and OEE, and *Bmp-4* expression was weaker than *Bmp-2* expression. *Msx-1* was not detected in the HERS cells, in contrast, strong *Msx-2* expression was detected in the HERS cells compared with the reaction of the dental pulp and dental follicle[3]. During root development, Shh is strongly expressed in the HERS, which suggests a function in root formation. Patched, the membrane receptor of Shh, and Gli1, a transcript activated by Shh, are also detectable in HERS[27,28]. Human HERS cells have been reported to express some stem cell markers, such as Bmi-1, Nanog, SSEA-4 and Oct3/4[29]. VIP-VPAC1 signaling pathway could directly promote proliferation of HERS cells and contribute to root formation[30].

Fate of HERS

During initial tooth-root formation, HERS cells undergo an extensive period of proliferation. At later stage, however, these epithelial cells decrease in number. When the first layer of dentin has been laid down, HERS loses its structural continuity and close relation to the surface of the root. These epithelial remnants are found in the periodontal ligament of erupted teeth and are called epithelial rests of Malassez, which present as an epithelial network of strands or tubules near the external surface of the root. Excluding ERM, there are other possible mechanisms put forward to explain the observed decrease in the number of HERS cells, including apoptosis and epithelial-mesenchymal transformation (EMT)[31-35].

Epithelial-mesenchymal transformation

Epithelial-mesenchymal transformation of HERS cells has been suggested[31]. Diverse studies showed that HERS cells participate in cementum formation through EMT[32,36,37]. Recently, many investigators have reported that HERS cells possess mesenchymal or cementoblastic characteristics both in vitro and in vivo. It was reported that human HERS cells expressed multiple types of cell markers, including epithelial molecules (amelogenin, cytokeratin, and E-cadherin), cementogenic/osteogenic molecules (BSP and OCN), mesenchymal molecule (vimentin), and epithelial-mesenchymal transition-associated molecules (β-catenin and N-cadherin). Both HERS cell clusters and individual HERS cells can co-express different markers, such as cytokeratin/N-cadherin and E-cadherin/osteocalcin. In addition, HERS cells expressed TGFβ-related molecules TGF-β1, TGFβ receptor I, and TGFβ receptor II. TGF-β1 signaling

pathway has been identified as one of the most important mechanisms that contribute to the epithelial-mesenchymal transformation process and was capable of inducing the epithelial-mesenchymal transformation of HERS cells through activating the PI3K/AKT pathway[36,38]. On the other hand, both TGF-β1 and FGF2 could induce the EMT of HERS cells by activating the MAPK/ERK signal pathway. FGF2 could induce HERS cells to cementoblast-like cells while TGF-β1 could induce periodontal ligament fibroblast-like cells[39]. Wnt3a expressed by HERS may contribute to the commitment of adjacent dental follicle cells toward a cementoblast/osteoblast phenotype[40]. Their finding is consistent with in situ studies demonstrating Wnt/β-catenin signaling in the region of the developing HERS in Axin2-lacZ reporter mice[41] and that a strong signal for β-catenin was detected within HERS at the apical root and pre-odontoblasts in wild-type mice[42].

CONCLUSION

In summary, there is increasing evidence from animal studies that HERS is not only a barrier between dental follicle and dental papilla cells but is also, through various signaling pathway and epithelial-mesenchymal interactions, involved in determining the shape, size and number of roots, the development of dentin and cementum or may act as a source of mesenchymal progenitor cells for cementoblasts in the periodontium through epithelial-mesenchymal transformation.

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국문초록

Hertwig's Epithelial Root Sheath의 운명과 의의

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발생과정 동안에 치아는 상피와 간엽간의 상호작용을 통해 형성되며, 이는 다양한 성장인자와 전사인자들에 의해 매개되는 것으로 알려져 있다. 치아머리의 형성과정은 치아상피의 증식과 분화에 의해 주로 조절되는데 반하여 치아뿌리의 형성과정에서 치아상피는 HERS라고 하는 구조로 나타난다. 현재까지 보고된 바에 따르면 이는 치아뿌리의 형태형성과 상아모세포의 분화, 그리고 EMT를 통한 백악모세포로의 분화에 있어서도 중요한 의미를 갖는 것으로 알려지고 있다. 본 논문에서는 현재까지 치아뿌리의 형성과정에서 알려진 HERS의 발생생물학적 이해를 살펴보고, HERS의 기능과 운명에 관한 최신 연구 동향을 고찰하여 치아뿌리와 치아주위조직의 형성에 HERS의 기여하는 바를 알아보고자 하였다.

주제어 : HERS, 치아뿌리, 백악질, 운명, 상피간엽전환