Clinical and Molecular Studies on Impacted Canines and the Regulatory Functions and Differentiation Potential of the Dental Follicle

Akademisk avhandling

Som för avläggande av odontologie doktorsexamen vid Sahlgrenska akademin, Göteborgs universitet kommer att offentligen försvaras i Föreläsningssal 3, Odontologen, Medicinaregatan 12D, fredagen den 15 december, klockan 9:00.

av

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Avhandlingen baseras på följande delarbeten

I. **Uribe P,** Ransjö M, and Westerlund A. Clinical predictors of maxillary canine impaction: a novel approach using multivariate analysis. European Journal of Orthodontics, 2017, 153–160. doi: 10.1093/ejo/cjw042.

II. **Uribe P,** Larsson L, Westerlund A, and Ransjö M. Gene expression profiles in dental follicles from patients with impacted canines. *Submitted for publication*

III. **Uribe P,** Plakwicz P, Larsson L, Czochrowska E, Westerlund A, and Ransjö M. Local patterns of regulatory factors expressed in human dental follicles. *Submitted for publication*

IV. **Uribe P,** Johansson A, Westerlund A, Larsson L, Magnusson C, and Ransjö M. Effect of soluble Silica on Cx43 gap junction communication and osteogenic differentiation in human dental follicle cells. *In manuscript*

**SAHLGRENSKA AKADEMIN
INSTITUTIONEN FÖR ODONTOLOGI**

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Abstract

Background: Impaction of the permanent maxillary canines, which is a common problem in dentistry, may require surgery and long-term orthodontic treatment. Until now, impaction has mostly been linked to physical obstructions and the direction of movement of the tooth. However, the molecular co-ordination of bone formation and bone resorption necessary for the eruption process, which is suggested to be regulated by the dental follicle, needs to be investigated further.

Aims: The overall objectives of this thesis were to determine which clinical factors are related to impacted canines, and to investigate the regulatory functions and differentiation potential of the dental follicle.

Patients and methods: The positions of impacted and normally erupting canines (orthopantograms), the skeletal variables (profile radiographs), and dento-alveolar traits (casts) were evaluated as potential predictive factors for impaction using a multivariate data analysis (N=90 patients). The gene expression profiles of bone-regulatory markers were determined by RT-qPCR and immunofluorescence staining of human dental follicles. Whole dental follicles (N=11) obtained from impacted canines, with or without signs of root resorption, and from control teeth (normal erupting teeth and mesiodens), together with the apical (N=15) and coronal (N=15) segments (processed independently), were analysed. *In vitro* osteogenic differentiation of human dental follicle cells (hDFC) was followed by the quantification of gene expression of osteoblast-phenotypic markers and Alizarin Red staining. Quantifications of the molecular permeability of gap junctional intercellular communication and of CX43 expression were performed with the dye parachute technique and flow cytometry, respectively. Next-generation sequencing and bioinformatics processing were used for the identification of differentially regulated genes and pathways involved in the differentiation of hDFC.

Results: Clinical variables related to the spatial location of the un-erupted tooth exert the strongest influences on impaction. However, they cannot be attributed to the cause of impaction, and they cannot be used as predictors. The RT-qPCR analyses revealed that the transcript levels for osteoclast-related markers (*M-CSF, MCP-1, RANKL*) were minimally expressed compared to those for osteoblastic markers (*RUNX2, COL-1, OSX, ALP, OCN*). No differential patterns of expression were identified between the impacted canines, with or without clinical signs of root resorption, or compared to the follicles from mesiodens or the normally erupting teeth. When the apical and coronal sections were analysed independently, significant differential expression was detected for the *RANKL* gene in the coronal part of the dental follicles, as compared with their corresponding apical parts. The induced expression levels of *RANKL* and *OPG* in cultured hDFC obtained from different patients were also significantly different. *CX43* was observed to be highly expressed in the follicular tissues, and its expression was increased when the cells were cultured in osteogenic medium, and even further enhanced when the cells were exposed to silica (Si). We found that multipotent stem cells residing in the dental follicle could be induced to differentiate towards an osteoblastic lineage under favourable *in vitro* conditions, resulting in regulation of the osteoblastic phenotypic markers (*RUNX2, OSX, BMP2, ALP,* and *OCN*) and active deposition of a mineralised matrix. In addition, Si enhanced osteogenic differentiation in combination with osteogenic induction medium, as revealed by increases in the expression of *CX43* and gap junction communication activity in the hDFC.

Conclusions: The results presented in the thesis reveal that clinical variables are influential, but not determinants, for tooth impaction. The dental follicle in the late pre-eruptive stage mainly expresses osteoblast-regulatory markers, whereas the levels of osteoclast-related markers are very low. Significant expression of CX43 and gap junction communication activity were detected, indicating an important role for these factors in the functional processes in the dental follicle. The significant upregulation of *RANKL* expression in the coronal part of the dental follicles suggests the importance of recruiting and activating osteoclasts, so as to form the eruption path through the alveolar bone. Moreover, the differential expression of induced *RANKL* in cultured hDFC may explain the diversity of events noted in the clinical setting during tooth eruption. Mesenchymal cells located in the dental follicle provide the optimal precursors, which can be cultured under *in vitro* conditions and further triggered with Si to differentiate towards an osteoblastic lineage.