

ANALYSIS OF GENE EXPRESSION IN ROOT REGION OF DEVELOPING TOOTH USING LASER CAPTURE MICRODISSECTION

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INTRODUCTION: Studies of tooth root development have been limited due to numerous difficulties in access to developing root region in mineralized tissue¹. Laser-capture microdissection (LCM) has been used on analysis of gene expression in mineralized skeletal tissues. However, the amount of total RNA of tissues in these reports was very low after decalcification^{2,3}. Thus, in this study, we described an approach of LCM without decalcification to identify various factors related with tooth root development. RT-PCR analysis was confirmed that the total RNA from two captured areas was sufficient in quantity and quality.

METHODS: Fresh mouse heads at E17.5 were dissected, embedded in TissueTek COT medium, and then frozen in liquid nitrogen. The tissue were sectioned at 7 μ m in a cryostat. As shown in Fig.1, the target areas (cervical loop and surrounding tissues) in the fresh sections were microdissected with a LCM (Arcturus Pixcell II). Section and staining conditions were assessed for the optimal retrieval of total RNA from microdissected cells. The mRNA expression levels of various signalling molecules were determined by RT-PCR.

RESULTS: Using the laser microdissection method, we dissected the cervical loop and a part of dental papilla from mouse tooth germs in both early and late bell stage. RNA was extracted from the dissected tissues, and RT-PCR for *Shh*, *Bmp4*, *Fgf10*, *Wnt10b*, and β -catenin was carried out. *Shh*, *Bmp4*, *Fgf10*, *Wnt10b*, β -catenin mRNA were expressed in the cervical loop and a part of dental papilla. The levels of β -catenin and *Bmp4* mRNA were much higher than those of other mRNAs in and around cervical loop. *Wnt10b* mRNA was weakly expressed in both areas.

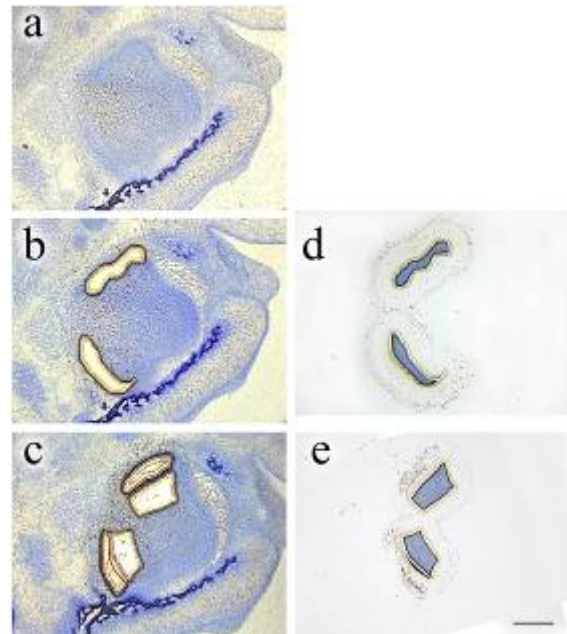


Fig. 1: Cells of the tooth germ at the late bell stage before (a) and after (b,c,d,e) laser microdissection (scale bar, 200 μ m). The white areas in fig.1b, c are areas that had been microdissected. Fig.1d,e are capture images.

DISCUSSION & CONCLUSIONS: We have succeeded in isolating total RNA by LCM method without decalcification and confirming the expression levels of several genes in order to characterize cervical loop and HERS during tooth root development. These findings showed the LCM-RT-PCR technique allowed study of gene expression in mineralized tissues such as tooth without decalcification.

REFERENCES: 1 M Aida, T Irié, T Aida, and T Tachikawa (2005) *J Dent Res.* 84(3):234-9. 2 YY Shao, L Wang, DG Hicks, RT Ballock (2006) *Lab Invest.* 86(10):1089-95. 3 S Yao, S Ring, WG Henk, GE Wise (2004) *Archives of Oral Biology* 49:451-6.