

TGF- β 1 INDUCES APOPTOSIS THROUGH SMAD SIGNALING-PATHWAY IN ODONTOBLASTS OF NFI-C NULL MICE

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INTRODUCTION: The critical roles of transcription factors and growth factors in tooth development especially for crown formation have been well documented. However, the molecular mechanism responsible for root development is not well defined. The NFI gene family encodes the site-specific transcription factors essential for the development of a number of organ systems. There are four NFI gene family members in vertebrates (*Nfia*, *Nfib*, *Nfic*, and *Nfix*). Our previous studies have demonstrated that nuclear factor I-C (*Nfic*) null mice developed short molar roots that contain aberrant odontoblasts and abnormal dentin formation. Based on these findings, studies were performed to uncover the underlying mechanism of *Nfic* function in odontoblasts during root formation.

METHODS: In this study, we investigated the expression of p-Smad2/3 and TGF β -RI in *Nfic* (-/-) mice using immunohistochemistry, RT-PCR and western analysis *in vivo* and *in vitro*. Second, we investigated if disruption of *Nfic* gene causes cell growth arrest and apoptosis of odontoblasts. We evaluated the cell proliferation and apoptosis by BrdU staining, MTT assay, flow cytometry, and TUNEL staining. Third, we tried to examine the molecular mechanism of cell growth arrest and apoptosis in *Nfic* (-/-) odontoblasts. Fourth, cDNA microarray was also performed for the identification of *Nfic*-related gene alteration in odontoblasts.

RESULTS: Initial studies demonstrated that disturbance of the *Nfi-c* gene increased both TGF β -RI and p-Smad2/3 expression in aberrant odontoblasts and pulp cells in the sub-odontoblastic layer *in vivo*, and primary pulp cells from *Nfic* (-/-) mice as well as MDPC 23 cells transfected with dominant negative transgene *in vitro*. Cell proliferation analysis of both pulp cells and HERS of the *Nfic* (-/-) mice revealed a significantly decreased proliferation activity compared to normal. Also, *Nfic* (-/-) primary pulp cells showed increased expression of p21 and p16, but a decreased cyclin D1 and cyclin B1 expression, strongly suggesting a cell

growth arrest when the *Nfic* gene function was disturbed. Analysis of apoptotic cells in the sub-odontoblastic layer of the pulp in *Nfic* (-/-) mice exhibited an increased apoptotic activity in *Nfic* (-/-) mice. Further, *Nfic* (-/-) primary pulp cells and *Nfic*-inactivated MDPC-23 cells increased not only the expression of Fas and FasL but also the activation of caspase-8 and -3, while the cleaved form of Bid was hardly detected. These results indicate that disturbance of the *Nfi-c* gene suppresses odontogenic cell proliferation and induces apoptosis of aberrant odontoblasts by up-regulating the expression of TGF β -RI and its downstream signaling molecules during root formation, contributing to the formation of short roots.

DISCUSSION & CONCLUSIONS: These results are expected to help better understand the molecular mechanism responsible for cell proliferation, differentiation and apoptosis in odontoblasts during tooth root development. However, more study will be needed to determine if *Nfic* is required in odontoblasts, the epithelial component of the root, or both, for root development using the conditional knockout allele of *Nfic*.

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