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Odontoblast-targeted Bcl-2 overexpression promotes dentine damage repair

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ABSTRACT

Objective: Bcl-2 is widely expressed in a developing tooth organ and regulates tooth morphogenesis. However, whether Bcl-2 is related to tooth damage repair is unknown yet. Using an odontoblast-targeted Bcl-2 overexpression transgenic mouse (Col2.3Bcl-2) and artificial cavity preparation as a model system, the relationship between Bcl-2 and reparative dentinogenesis is investigated in this study.

Methods: The odontoblastic-like cell cultures derived from mouse molar pulps were established. The expression of transgenic human Bcl-2 (hBcl-2) and endogenous mouse Bcl-2 (mBcl-2) and mouse Bax (mBax, a Bcl-2 antagonist) was detected in vivo and in vitro by Western blot and immunocytochemistry, respectively. Basal level and artificial cavity-induced odontoblast apoptosis was detected by the Deoxynucleotidyl Transferase (TdT) dUTP Nick End labelling (TUNEL) technique. Reparative dentine formation induced by artificial cavity drilled to a half dentine thickness on mesial cervical region of mandibular first molars 2, 4, and 6 weeks post-op was evaluated histologically and via micro-CT.

Results: The transgenic hBcl-2 was stably expressed in odontoblasts of the transgenic animals without interference with the expression of mBcl-2 and mBax. Basal level as well as artificial cavity- induced odontoblast apoptosis was prevented by the transgene. Compared to the wild type, the transgenic animals produced reparative dentine with significantly higher mineral density 6 weeks after the operation.

Conclusions: Bcl-2 overexpression prevents odontoblast apoptosis and promotes dentine damage repair, indicating that genetic manipulation of Bcl-2 may be a novel strategy to maintain the vitality and function of dentine-pulp complex under detrimental mechanical stimuli.

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Abbreviations: α-MEM, alpha-minimum essential medium; BCA, bicinchoninic acid assay; Bcl-2, B-cell lymphoma-2; hBcl-2, human Bcl-2; mBcl-2, mouse Bcl-2; mBax, mouse Bcl-2-associated X protein; βGP, beta-glycerophosphate; BMP, bone morphogenetic protein; BSA, bovine serum albumin; DTT, dithiothreitol; EDTA, ethylenediaminetetraacetic acid; E18, embryonic day 18; FGF, fibroblast growth factor; HRP, horseradish peroxidase; IGF, insulin-like growth factor; μCT, micro X-ray computed tomography; Msx, msh homeobox; PCR, polymerase chain reaction; RT, room temperature; SDS-PAGE, sodium dodecyl sulphate-polyacrylamide gel electrophoresis; TdT, terminal deoxynucleotidyl transferase; TGFβ, transforming growth factor beta; TTBS, Tween 20 in tris buffered saline; TUNEL, deoxynucleotidyl transferase dUTP Nick end labelling; ++, wild type; tg/tg, homozygous transgenic.

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1. Introduction

The development of teeth is under strict genetic control and is regulated by inductive interactions between oral epithelium and cranial neural crest-derived mesenchymal cells.[1,2] The reciprocal and sequential epithelial–mesenchymal interactions instruct a tooth germ to proceed through bud, cap, and bell stages, before the mesenchymal odontoblasts and epithelial ameloblasts differentiate terminally and deposit the organic matrices of dentine and enamel, respectively.[3,4]

Normally three types of dentine may be formed throughout the life of a tooth. Primary dentine is produced by odontoblasts at an active secretory phase during the formation of the crown and root(s) of the tooth. After completion of root formation, physiological secondary dentine is secreted by odontoblasts at a continuing, albeit much reduced rate. Under environmental stimuli, odontoblasts may generate tertiary dentine (reactionary or reparative variants) at the site of injury. In the case of mild injuries, such as non-cavitated or slowly progressing caries, mild abrasion/erosion, odontoblasts focally up-regulate their secretory activity and deposit reactionary dentine. However, more intensive stimuli, such as advanced dental caries, cavity preparation, and therapeutic or restorative dental materials, may induce odontoblast apoptosis and reparative dentine formation by odontoblastic-like cells derived from progenitor cells within the pulp.[5–8] Reactive dentine shares many similarities to the primary and secondary dentine and can effectively oppose exogenous detrimental stimuli to protect the pulp.[9] Reparative dentine is more diversified, which contains both atubular and tubular dentine.[10] In clinical scenery, tooth damage repair is demonstrated as a mixture of reactionary and reparative dentine, which is indistinguishable at the in vivo level, nor from a biochemical and molecular point of view.[8]

Dentinogenesis is under the regulation of a variety of growth factors and transcription factors. Strong parallels exist between many tooth developmental events and dental damage repair. BMPs, TGFβs, FGFs, IGF, Msx-1 and -2, c-jun, and jun-B are all found to regulate differentiation and functions of odontoblasts.[11–14] Sequestration of these factors within dentine matrix will allow their release in carious and injured teeth, due to matrix demineralisation and dissolution. These factors will further act as both mitogens and chemotactic factors for pulp cells to signal reparative process at the injured sites.[15–19]

In addition to growth factors and other relevant mediators, apoptosis seems to play a pivotal role in developmental and damage-repair related dentine remodelling. Apoptosis in odontoblasts has been shown in rodent incisors and human molars of different ages.[20] Massive programmed cell death may be the strategy employed by odontoblasts to cope with reduced pulp chamber size after secondary dentine deposition.[20,21] For teeth under violent stress, odontoblasts adjacent to the injured sites will undergo apoptosis and replaced by pulp progenitors-derived odontoblastic-like cells to elaborate reparative dentine.[22–26] Amongst numerous apoptosis regulators, Bcl-2, an anti-apoptotic protein, is expressed at various stages of a developing tooth.[27–29] In odontoblasts underlying cavity preparation, Bcl-2 expression was found to be increased significantly.[24] These observations suggest that regulation of odontoblast apoptosis by Bcl-2 may contribute to dentine homeostasis and damage-related dentine remodelling. However, how Bcl-2 is related to these physiological and pathological processes is not entirely clear yet.

Using a Col2.3Bcl-2 transgenic mouse, in which hBcl-2 is driven by the 2.3 kb fragment of rat type I collagen promoter, we have previously shown that primary dentinogenesis is impaired by odontoblast-targeted Bcl-2 overexpression, as demonstrated by much thinner and less dense dentine in the transgenics compared with age-matched wild type animals.[30] In the present study, how dentine repair is affected by Bcl-2 was investigated with the same transgenic animals, using artificial cavity preparation as a model system for intense mechanical stimulus. These studies are anticipated to expand our fundamental understanding on tooth developmental biology and regeneration, and ultimately lead to novel therapeutic interventions for dental developmental anomalies and damage repair.

2. Materials and methods

2.1. Animals

The Col2.3Bcl-2 transgenic mice and their wild type littermates were imported from University of Connecticut Health Centre. The methods of creating the transgenic mice and a PCR-based genotyping were described previously.[31] Briefly, transgenic mice were identified using PCR of ear punch isolated genomic DNA with 5′-tgaagtcaacatgcctgcc and 3′-ctctaaaggtgcggcttcct primers that produce a 670-bp product specific to the 3′ untranslated region of hBcl-2. All animal-related experiments were approved by the Centre for Laboratory Animal Medicine and Care at the University of Texas Health Science Centre at Houston.

2.2. Pulp cell cultures

Coronal molar pulps of 5-day-old mice were isolated and digested with 0.05% trypsin and 0.1% collagenase P at 37 °C for 50 min. Cells were plated onto NALGE-NUNC Lab-Tek 4-well chamber slides (Nalge Nunc International, Rochester, NY) at a density of 10^4 cell/cm^2 in α-MEM containing 10% foetal bovine serum (FBS), 100 U/ml of penicillin and 100 μg/ml of streptomycin. After confluence (normally on day 7), the cells were induced to differentiate with 10^{-8} M dexamethasone, 8 mM β-glycerophosphate (βGP), and 50 μg/ml ascorbic acid in α-MEM for 1 day, then the medium was switched to α-MEM + 10% FBS + 4 mM βGP + 50 μg/ml ascorbic acid, and was changed every other day thereafter. The day of plating pulp cells was counted as day 1, and on subsequent days 7, 14, and 21, immunocytochemistry or TUNEL assay was performed on the cultures as mentioned below.

2.3. Immunocytochemistry

Pulp cells were fixed with 70% ethanol at −20 °C for 2 h. The samples were air dried, and endogenous peroxidase activity