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Short communication

BMP-7 gene transfer to inflamed ferret dental pulps

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In vivo and *ex vivo* gene transfer are being developed for localized skeletal regeneration. These strategies for tissue regeneration were tested in an adult ferret model of vital pulp therapy. In this model a reversible pulpitis was induced first. Then after 3 d, the pulps were directly infected with recombinant virus or implanted with *ex vivo* transduced autologous dermal fibroblasts. The genome of the recombinant adenovirus contained a full-length cDNA encoding mouse bone morphogenetic protein (BMP)-7 (AdBMP7) or bacterial β-galactosidase cDNA (AdlacZ). The BMP-7, but not lacZ, *ex vivo* transduced dermal fibroblasts induced reparative dentinogenesis with apparent regeneration of the dentin-pulp complex. *In vivo* infection with AdBMP-7 failed to produce reparative dentin in all cases. *Ex vivo* gene transfer of BMP-7 may be an effective method for inducing dentin regeneration in teeth with reversible pulpitis.

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The dentin-pulp complex (DPC), comprising odontoblasts and their extracellular matrix (dentin), is functionally important for pulp homeostasis (1). Tertiary dentin matrices (formed in response to specific stimuli in adult teeth) are categorized as reparative or reactionary. Reparative dentin (RD) is a tertiary dentin matrix formed by new odontoblast-like cells in response to a specific stimulus after loss of the primary odontoblasts (2). Clinical and experimental studies reveal that RD forms in adult pulps after pulp exposure in germ-free rats (3), monkeys and humans (4–7). However if too many odontoblasts are lost, tertiary dentinogenesis and DPC regeneration fail to occur. It is well known that conventional medicaments such as calcium hydroxide fail to induce a reparative response if the pulp exposure is too large. This phenomenon may be analogous to critical size bony defects (8–10).

Bone morphogenetic proteins (BMP) are a family of pleiotropic signaling molecules critically involved at various stages in the formation of a variety of tissues and organs including bones and teeth (11, 12). In addition as recombinant proteins, they have the remarkable ability to induce bone or RD formation when applied directly to adult dermis, muscle or bone (13) or dental pulp respectively (14–16). Most data indicate that connective tissue repair is mediated by resident stromal cells adjacent the wound (17). Therefore it is likely that mature adult pulps contain cells capable of producing RD in response to a BMP signal (18, 19). In support of this concept is the fact that dentin, like bone, contains BMP activity (20, 21). Pulp fibroblasts express the BMP receptors BMPR-IA, -IB, ActR-1 and BMPR-II mRNA transcripts (22).

Exogenous recombinant BMP-2 and -4 induce RD in dogs (16) as does recombinant human BMP-7, but not TGF-β (Rutherford, unpublished data), in ferrets (23) and monkeys (14, 15). However, BMP-7 protein therapy failed to induce reparative dentinogenesis in experimentally inflamed ferret pulps containing cells that expressed the appropriate BMP receptor transcripts (23). Direct application of BMP to pulp, while efficacious in stimulating a reparative response, may not be a clinically useful method for induction of DPC regeneration. Therefore we tested the hypothesis that BMP-7 gene transfer would induce DPC regeneration in inflamed dental pulps.

Material and methods

To test this hypothesis, we constructed and studied the osteogenic capacity of a recombinant adenovirus containing the full-length mouse BMP-7 gene (AdBMP7) as described (10, 24, 25). In the current study, inflammation was induced in male adult ferret dental pulps by direct injection of bacterial lipopolysaccharide and evaluated as described (23). The inflamed teeth were subsequently infected directly *in vivo* with AdBMP7 suspended at 10^8 pfu/μl in a type I collagen thermoset hydrogel (RD Bioscience, Waltham, MA, USA). Virus free carrier and non-inflamed pulps served as controls (4 teeth per group). Alternatively autologous fibroblasts were cultured from dermal biopsies of 3 adult male ferrets (26) and infected *ex vivo* with AdBMP7 ($n=4$) or AdlacZ (negative control, $n=4$) as described (10, 25). One million *ex vivo* transduced (BMP-7 and lacZ) autologous fibroblasts per group were suspended in 10 μl of the collagen thermoset hydrogel and injected into inflamed dental pulps (4 teeth per group). An equal volume of cell-free carrier was

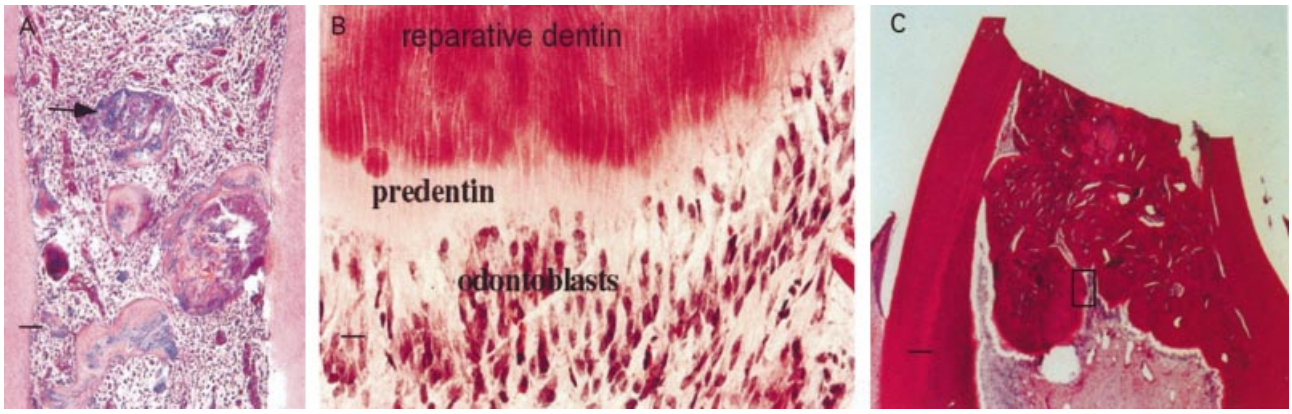

Fig. 1. Histomicrographs of inflamed adult ferret teeth treated by *in vivo* infection of AdBMP-7 (A) or *ex vivo* infection and implantation of BMP-7 transduced autologous cultured dermal fibroblasts (B, C). A) Small islands (solid arrow) of a poorly developed mineralizing matrix are seen in a hyperemic dental pulp (50×, bar = 40 μ). B) A mass of reparative dentin lined by a layer of predentin and odontoblasts are displayed (100×, bar = 80 μ). C) The mass of reparative dentin is incompletely mineralized after 30 days (25×, bar = 20 μ). The section displayed in (B) was taken from a parallel section to the one depicted in (C) as outlined. Preparations were stained with H&E and photographed with a Spot Digital Camera mounted on an Olympus B × 60 microscope. The images were captured in Adobe Photoshop (v. 5) and adjusted for brightness and contrast only.

also injected ($n = 4$). The teeth were harvested after 30 d and processed for histologic and histomorphometric evaluation (23). Calcium hydroxide was not attempted in this study, because previous attempts had failed in this model (Rutherford, unpublished data).

Results and discussion

At best, small amounts of poorly organized mineralizing masses formed in some of the teeth to which virus were directly injected (Fig. 1A, solid arrow). In contrast, RD with an apparently regenerated DPC formed in all of the teeth receiving the *ex vivo* BMP-7 transduced autologous dermal fibroblasts (Fig. 1B, C). Organized layers of RD, predentin and odontoblast-like cells separated the pulp from new dentin. In all these cases the mass of RD was intact, partially contiguous with the adjacent dentinal walls, and the extent of RD was limited to the crown. Histomorphometric calculations (14, 27) suggested that on average approximately 6 μl of RD formed per tooth. Longer incubation times are planned to determine if the mass of reparative dentin coronal to the DPC continues to mature as was the case for recombinant BMP protein induced reparative dentinogenesis in monkeys (15).

These data reveal that *ex vivo* mediated BMP-7 gene transfer induces reparative dentinogenesis and DPC regeneration in inflamed dental pulps. This result (Fig. 1B) is substantially different than the response of inflamed dental pulp to exogenous recombinant BMP-7 protein (23) or *in vivo* BMP gene transfer (Fig. 1A). These methods likely fail because, in the presence of inflamed tissue, insufficient active BMP-7 protein is available when added exogenously and too few cells are infected by AdBMP7. It is likely that the implanted transduced fibroblasts secrete BMP-7 *in vivo* upon implantation as has been observed in our osteogenic assays (25). The amount and duration of BMP-7 secretion appears adequate to stimulate resident cells in inflamed tissue. Of interest is that any innate or acquired immunity to the adenovirus infected cells doesn't prevent the formation of reparative tissue. No inflammation is evident in the pulp deep to the DPC (Fig. 1B) by 30 d. Extensive studies of the immune response to implantation of syngeneic adenovirus infected cells are underway.

It will be important to determine the fate of the transduced implanted cells. *Ex vivo* BMP-7 transduction induces the osteoblastic conversion of fibroblasts *in vivo* (25) so that the induced bone is a chimera of implanted and graft-site cells (10). Therefore it is possible that in the experiments reported here, the implanted BMP-7 transduced cells directly contribute to the production of tertiary dentin matrix as well as induce host cells by a paracrine mode of action. Experiments exploring this possibility are being planned.

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