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Gene therapy strategies for craniofacial tissue engineering

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Ex vivo gene therapy is a rapidly developing approach for bone tissue engineering. Pre-clinical studies have consistently shown high success rates of this therapy for healing critical-sized segmental bone defects. Its efficacy has not been tested yet, however, in radiated bone defects. Since bone reconstruction in the head and neck region is frequently performed in the context of pre- or post-operative radiation, the effectiveness of this regenerative approach in radiated defects needs to be determined prior to considering application to patients. The primary objective of this study is to determine the effects of radiation on *ex vivo* gene therapy-directed osteogenesis in critical-sized cranial defects.

We develop an integrated tissue engineering strategy to regenerate bone by combining a molecular medicine approach with *ex vivo* gene therapy using an adenovirus driving the expression of BMP-7. The major groups consisted of rats treated with either a single 12 Gray (Gy) dose of radiation 2 weeks after surgery, a single 12-Gy dose of radiation 2 weeks before surgery, or no radiation. A 9-millimeter critical-sized calvarial defect was created and periosteum was excised. Within each of these groups, defects were treated with either an inlay calvarial bone graft or syngeneic dermal fibroblasts transduced *ex vivo* with an adenovirus containing the cDNA for BMP-7. One group also received daily subcutaneous injections of recombinant human parathyroid hormone (PTH [1-34]) (60 mg/kg) for 6 weeks. Transduced fibroblasts were seeded on a collagen sponge for delivery to the wound. Non-radiated defects were harvested 4 weeks later for both the bone graft and gene therapy treated groups. Defects treated with post-operative radiation were harvested 4 weeks after the radiation dose. Defects treated with pre-operative radiation were harvested 4 weeks after bone graft surgery and those treated with gene therapy were harvested either at 4 or 8 weeks after the surgery. Gross inspection, micro-CT and histomorphology were used to evaluate wound healing.

Results: None of the bone grafts had gross or histologic evidence of healing at the wound margins in the radiated or non-radiated defects. The non-radiated gene therapy treated defects revealed gross and histologic near-100% bone regeneration by 4 weeks after surgery. Irradiated animals treated with anabolic doses of PTH demonstrated significantly increased percentage bone volume/total defect volume than *ex vivo* gene therapy treated controls (60.36±5.67%)(PTH) versus 41.16±9.71% (non-irradiation control), ($p<0.05$). Post-operative radiation therapy significantly limited tissue regeneration by the combination of BMSC cells and PTH treatment (46.21±8.30% versus 39.16±1.36%). However, in pre-operative irradiation groups, very poor bone regeneration attained even by the combination of PTH treatment (24.15±2.83% versus 17.94±1.93%). Bone mineral density was also significantly increased by the addition of PTH treatment in both the post-operative irradiation and non-irradiation groups.

Conclusions: These results indicate that both pre- and post-operative radiation have detrimental effects on bone regeneration induced by BMP-7 *ex vivo* gene therapy. The characteristics of these negative effects were very different, however, dependent on the timing of radiation. The combination therapy of *ex vivo* gene therapy and anabolic PTH significantly enhanced healing even in irradiated bone defects. These findings have significant implications for translating this tissue engineering approach to patients with cancer-related segmental bone defects.

The authors have no conflict of interest.

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