NOGGIN, A BMP ANTAGONIST, ENHANCES REGULATED BONE FORMATION AND BONE HEALING ELICITED BY INDUCIBLE BMP4 EXPRESSION

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Introduction
Regulated therapeutic gene expression is required for many gene therapy applications, particularly in stem cell-based gene therapy, due to the cells potential for long-term persistence and multipotent differentiation. To date, one of the most intensively studied inducible gene expression systems comprises the tetracycline (tet)-controlled gene and its corresponding synthetic promoter, a system that researchers have utilized for either tet-on (gene expression activated by the presence of tet) or tet-off (gene expression activated in the absence of tet) applications. Researchers have developed retroviral vectors containing tet-on- or tet-off-controlled therapeutic genes with varying degrees of success. We initially tried to optimize the most promising design to date—the self-inactivating (SI) tet-on retroviral vector—to derive a retroviral vector suitable for regulated bone morphogenetic protein (BMP) gene therapy to improve bone healing. We then developed a new strategy which involved the use of noggin, a BMP antagonist, to stringently regulate the therapeutic effects of the inducible BMP4 expression.

Materials and Methods
Generation of retroviral vectors and transduction of cells. Standard molecular biological techniques were used to construct a series of 6 SI-retroviral vectors expressing human BMP4. To facilitate the screening of these vectors, an enhanced green fluorescent protein (EGFP) gene was inserted downstream of the BMP4 cDNA with an internal ribosome entry site (IRES). To determine the levels of gene expression, the vector DNA was transfected into 293 cells using calcium-phosphate precipitation. The levels of EGFP expression were determined using quantitative fluorescent microscopy. To generate replication defective retroviruses, the vector DNA was transfected into packaging cells. The vector DNA was transfected into 293 cells using calcium-phosphate precipitation. The levels of EGFP expression were determined using quantitative fluorescent microscopy. To generate replication defective retroviruses, the vector DNA was transfected into packaging cells. The vector DNA was transfected into 293 cells using calcium-phosphate precipitation. The levels of EGFP expression were determined using quantitative fluorescent microscopy.

Discussion and Conclusions
We have identified the optimal design for a self-inactivating, tet-on retroviral vector that can efficiently transduce MDSCs and promote bone formation and bone healing through inducible BMP4 gene expression. More importantly, we have developed a novel strategy to improve the therapeutic outcome of regulated BMP4 expression through the use of a specific BMP antagonist—noggin. We believe this vector system has great potential for gene therapy to improve bone regeneration. The novel concept of improving regulated gene therapy via the co-delivery of a specific antagonist also could be applied to other systems in which stringent regulation of the therapeutic effects of an inducible gene is necessary.

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