INTRODUCTION: Disorders of heterotopic ossification occur in a wide variety of disease processes, and prevention regimens using indomethacin and radiation therapy have had varying success. However, for individuals with more severe forms of heterotopic ossification such as fibrodysplasia ossificans progressiva (FOP), conventional interventions have failed to halt progression of the disease. Although the genetic causes of FOP and other disorders of heterotopic ossification remain unknown, overexpression of bone morphogenetic protein-4 (BMP4) and underexpression of the BMP antagonist noggin may have important inductive roles in the pathogenesis of such disorders.

The purpose of this study was to develop an effective gene therapy approach for the prevention of BMP mediated heterotopic ossification that would be applicable in principle to the treatment of patients with FOP. To achieve this goal, we used an adenovirus-mediated gene transfer of noggin, a potent secreted extracellular antagonist of the osteogenic morphogen, BMP4.

METHODS: A mouse model of induced heterotopic osteogenesis was developed using an injectable osteoconductive material (carrier) impregnated with BMP4. This model permits the definitive identification of the stages of endochondral bone formation (Figure 1). The abdominal musculature of 32 C57/b6 mice (with IACUC review board approval) was injected on one side of the midline with 250µl of carrier alone, and on the contra-lateral side with 250µl of carrier combined with recombinant human BMP4 at a final concentration of either 50µg/ml or 12.5µg/ml. Both doses of BMP4 were previously determined to consistently produce heterotopic ossification in a dose-dependent manner. Half of the animals were pretreated for 4 days with 1x10¹⁵ viral particles/mouse of recombinant adenovirus carrying the human noggin gene (AdhNgdeltaB2, a modified noggin gene engineered to increase protein bioavailability). Implants were recovered at 7 and 14 days after injection. Standard histological techniques were used to evaluate the histologic stages of bone formation and to identify specific cell types present in the tissue.

RESULTS: Experimental and control implants were recovered from all animals. The implants containing BMP4-induced an aggressive, fibroproliferative lesion with early cartilage formation at 7 days (Figure 2a), and heterotopic ossification at 14 days. However, in animals treated with noggin-containing adenovirus, the implants with BMP4 demonstrated a minimal, mixed inflammatory cell infiltrate at 7 days (Figure 2b) and a thin pseudocapsule several cell layers thick surrounding the unresorbed plug at 14 days, indistinguishable from carrier implants with no BMP.

DISCUSSION: This study demonstrates that the delivery of noggin through a gene therapy approach successfully prevents BMP4 induced heterotopic ossification in a mouse model. It provides proof-of-concept that a secreted morphogen antagonist can be produced in vivo and act systemically to prevent BMP4-mediated heterotopic ossification that has been difficult to prevent. Numerous obstacles need to be overcome before the application of noggin gene therapy could be considered for the treatment of patients who have FOP and other disorders of heterotopic ossification. These include the development of safe and effective viral vectors and inducible promoters for the systemic and durable delivery of genes in humans and ultimately the development of improved animal models based on knowledge of the molecular genetics of FOP and other disorders of heterotopic ossification.

IMPLICATIONS FOR THE TREATMENT OF FIBRODYSPLASIA OSSIFICANS PROGRESSIVA AND OTHER DISORDERS OF ECTOPIC OSSIFICATION IN HUMANS.

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