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Promotion of functional heterotrimeric type I collagen via transfection in osteogenesis imperfecta fibroblasts

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Osteogenesis imperfecta (OI) is a heritable disorder due to mutations in type I collagen. Normal type I collagen forms a heterotrimeric protein comprised of two proα1(I) chains and one proα2(I) chain [$\alpha1(I)_2\alpha2(I)$]. The osteogenesis imperfecta murine (*oim*) model mouse contains a single nucleotide deletion in the proα2(I) gene (COL1A2) resulting in non-functional proα2(I) chains and production of homotrimeric type I collagen containing three proα1(I) collagen chains, [$\alpha1(I)_3$], resulting in small body size, increased bone fragility and altered bone mineralization.

The overall goal of this study is to correct the *oim* defect by introducing normal COL1A2 genes into *oim* cells. *Oim* dermal fibroblasts were transfected with a series of COL1A2 gene constructs containing the full-length murine proα2(I) collagen cDNA driven by various lengths of the murine COL1A2 promoter (1.5kb, 3.0kb, and 6.0kb) along with a COL1A2 enhancer. These DNA constructs were cotransfected with pcDNA3 containing a neomycin resistance gene, which allows for selection of stably transfected cell lines. Various assays have been developed to monitor proa2(I) collagen expression at the DNA, RNA and protein levels. A PCR assay was used to confirm genomic incorporation of transgenic COL1A2 gene constructs and an RT-PCR assay used to confirm expression of normal proα2(I) collagen mRNA. Denaturing urea-SDS polyacrylamide gel electrophoresis along with Western blotting analyses using anti-murine α1(I) and α2(I) collagen antibodies were used to confirm normal proα2(I) collagen expression at the protein level as well as its incorporation into normal heterotrimeric type I collagen.

All the necessary tools have been established for evaluating the efficacy of transfection. Currently, the first series of stably transfected *oim* cell lines are being expanded for analyses as described above. Although this study is aimed at 'fixing' the *oim* mutation via gene therapy, valuable data will also be collected regarding the effectiveness of the variable length promoter regions in enhancing the expression of the normal COL1A2 gene.