ADENOVIRAL TRANSFER OF A MOUSE COL1A2 INTO MURINE BONE MARROW STROMAL CELLS DEFICIENT IN THE COLLAGEN GENE RESTORES THE SYNTHESIS OF NORMAL TYPE I COLLAGEN

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Introduction

Osteogenesis imperfecta (OI) is a heritable disorder of connective tissue whose hallmark is bone fragility. Most forms of OI result from mutations in the genes that encode proα1 and proα2 chains of type I collagen. At present there is no cure for the disease, since the disease is genetic, pharmacological agents are not expected to be effective. In the present investigation, the feasibility of gene therapy as a cure for the disease was investigated by determining the potential of bone marrow stromal cells from a mouse model of OI (oim) deficient in proα2(I) gene to be transduced with the collagen gene and to express the gene in vitro and in vivo.

Methods

Vector construction: The recombinant adenoviral vector is a replication deficient type 5 adenovirus that lacks the E1 and E3 loci. The murine proα2(I) cDNA cloned in SP72 vector was released from plasmid by Hind III and Bam HI. The collagen gene was inserted in the place of the E1 region and the expression is driven by the cytomegalovirus. High titre suspensions of the recombinant adenovirus were prepared by transfection of Cre 8 cells with a Ψ 5 helper virus DNA and pAdlox collagen proα2(I) cDNA construct digested with sfi I. The cells were subjected to freeze/thaw cycles and the recombinant virus was purified and amplified. The final purification of the virus was done using cesium chloride gradient ultracentrifugation.

Bone marrow stromal cell isolation: Bone marrow stromal cells were established by flushing the marrow from the femurs of oim mice into T-25 flasks. The cells were maintained in culture without disturbance for 7 days and the non-adherent cells were removed. The adherent cells were maintained in culture with media changes every 2 to 3 days as described previously (1).

Transduction of bone marrow stromal cells: Bone marrow stromal cells from oim mice were plated in 6 well plates in DMEM supplemented with 10% FBS and 50 μg/ml ascorbic acid. At confluence the media were removed and cells were transduced by adding an adenovirus containing the mouse proα2(I) cDNA in 1ml of serumless media. The transduced cells were transferred to regular media supplemented with 50 μg/ml ascorbic acid.

Analysis of collagen synthesis: After 16 h, the transduced cells were transferred to serumless media supplemented with 50 μg/ml β-aminopropionitrile, 50 μg/ml ascorbic acid and 10 μCi/ml of [^{3}H]proline. The cells were incubated at 37°C in 5% CO2 for 24h and the media and cell layer were harvested, adjusted to 0.5M acetic acid and treated with pepsin at 100 μg/ml for 4 h at 4°C. The collagen resistant chains were precipitated by addition of NaCl and dialyzed against NH_4CO_3. The dialysates were freeze-dried and analyzed by SDS-PAGE followed by autoradiography.

Alkaline phosphatase activity: The transduced cells were plated in 48 well plates and treated with 100ng/ml of rhBMP-2 every the other day for 6 days. Cells were subjected to freeze thaw cycles and the lysates were analyzed for ALP activity.

Expression of α2(I) chain in vivo. Oim mice were injected under the skin with the adenovirus expressing the proα2(I) chain. The injected site was marked with ink for identification. The opposite side of the mice skin were injected with saline and served as controls. Pieces of skin were harvested from the virus injected and saline injected sites and the collagens were extracted by mild pepsin digestion. The extracted collagens were analyzed by SDS-PAGE.

Results:

The established bone marrow stromal cells from oim mice synthesized α1(I) homotrimers as described previously (2). The cells deficient in α2(I) chain expressed ALP activity when treated with rhBMP-2. The bone marrow stromal cells from normal littermates synthesized α1(I) and α2(I) heterotrimers. Oim bone marrow stromal cells transduced with the adenovirus containing the mouse proα2(I) cDNA synthesized type I collagen consisting of α1 and α2 chains in a 2:1 ratio (fig. 1). The amount of type I collagen synthesized by the oim transduced cells was equivalent to that of the normal control when the appropriate MOI that gave the highest expression of α2(I) chain without affecting the cells was used. The amount of α1(I) and α2(I) heterotrimers synthesized in vitro was regulated by the concentration of the virus (MOI) used to transfect the cells. When a higher concentration of the virus was used enough proα2(I) chains were synthesized and presumably moped up all the α(I) chains resulting in a type I collagen heterotrimer consisting of α1 and α2 chains in a ratio of 2:1. When the virus was injected in the mouse skin, the tissue harvested 7 days after injection demonstrated presence of α2(I) chains suggesting synthesis of type I collagen consisting of α1 and α2 heterotrimers at the injected site. The saline injected site did not show any presence of α2(I) chains.

The transduced cells expressed ALP activity when they were treated with rhBMP-2 suggesting that transduction of the cells with the collagen gene did not affect the osteogenic potential of the cells.

Discussion

We have shown that transduction of bone marrow stromal cells deficient in the proα2(I) gene rescues the cells in vitro. These data demonstrate that transduction of bone marrow stromal cells with the collagen gene is possible and that the gene is expressed by the cells at high efficiency. These data show promise for the development of gene therapy for OI null mutations and also for studying the OI pathology.

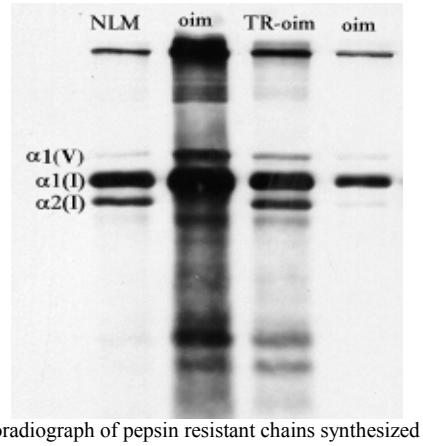

Fig. 1. Autoradiograph of pepsin resistant chains synthesized by NLM (normal mouse), oim mouse (oim) and (TR-oim) oim cells transduced with adeno-COL1A2., oim= oim cells transduced with very high concentration of the virus which killed the cells.

References:1. Oyama et al. Gene Therapy 6: 321-329, 1999. 2. Balk et al. Bone 21: 7-15, 1997.

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