Introduction: Adipose-derived stem cells (ADSCs) have shown the potentials to differentiate toward the chondrogenic phenotypes with growth factors [1]. But the chondrogenic differentiation with proteomic growth factor needs a prolonged duration of in vitro culture, which would obviously delay the treatments to patients. Growth and differentiation factor 5 (GDF5) is a member of the TGF-beta superfamily that is best known for its role in early chondrogenesis and joint formation. GDF5 up-regulates chondrocyte-specific expression of type II collagen and accumulation of extracellular matrix glycosaminoglycan (GAG), indicating that GDF5 could promote the differentiation of mesenchymal stem cells into chondrocytes [2]. In addition, GDF5 initiates chondrogenic differentiation of bone marrow-derived stem cells [3]. This study was designed to assess the chondrogenic differentiation potentials of ADSCs transduced with adenovirus GDF-5 in order to lay a foundation for the future cell-based therapy in the damaged cartilage repair.

Materials and Methods: Construction of Ad-GDF5: A 1.7 kb human GDF5 cDNA sequence was used to generate the adenoviral GDF5 vector (Ad-GDF5) with AdEasy adenoviral vector system.

Chondrogenic differentiation of ADSCs: ADSCs were prepared from the inguinal fat pads of 8-week-old Fischer 344 rats. High density pellet cultures of ADSCs were transduced with Ad-GDF5, or treated with recombinant human GDF-5 (rhGDF5) and transforming growth factor-beta 1 (rhTGF-β1).

Histology analysis of the pellet cultures: The pellet cultures from each culture condition were harvested and fixed in 10% buffered formalin and embedded in paraffin. Samples were then cut into 5μm sections, rehydrated and stained with Safranin-O for detection of proteoglycan.

Biochemistry analysis: Sulfated glycosaminoglycan (sGAG) levels were measured spectrophotometrically after incubation with 1, 9-dimethylmethylen blue chloride (DMMB) dye and normalized to total DNA, as measured fluorometrically using the bisbenzimidide Hoechst 33258 DNA Quantitation Kit.

Gene expression analysis: Collagen I, Collagen II, Aggrecan and Collagen X gene expression patterns in the process of chondrogenic differentiation were analyzed with real-time PCR.

Immunohistochemistry staining: For detection of collagen II, the pellet sections were incubated with Arthrogen-CIA collagen II monoclonal antibodies and then developed with DAB. For visualization of collagen X and aggrecan, sections were incubated with either rabbit anti-rat collagen X polyclonal antibody or rabbit anti-rat aggrecan polyclonal antibody, followed by incubation with Texas red (for collagen X) or FITC (for aggrecan) conjugated secondary antibody.

Results: Western blotting revealed that two human GDF-5 protein forms, full-length precursor and mature monomer, were detected in the culture medium from Ad-GDF-5 infected ADSCs. ELISA results demonstrated GDF-5 production peaked at 1 week after infection and higher viral multiplicity of infection (MOI) led to increased GDF-5 synthesis, which reaching the plateau at 150 MOI. ADSCs transduced with Ad-GDF5 (MOI=150), or induced with rhGDF5 (100ng/ml) and rhTGF-β1 (10ng/ml) in pellet culture demonstrated robust chondrogenic differentiation. The type II collagen and aggrecan mRNA transcription were significantly increased upon the viral or protein based GDF-5 treatment from 1 week to 3 weeks compared with plain chondrogenic media (Fig 1), and their proteins were distributed throughout the matrix in pellets (Fig 2). The proteoglycan deposition, as assessed by Safranin-O staining, distinctively increased, which is consistent with the upregulated GAG content in the quantitatively biochemical assay.

Discussion: In the present study, we demonstrated that adenoviral-mediated expression of GDF5 can serve as an effective means of protein delivery to induce chondrogenesis of rat adipose tissue-derived stem cells (ADSCs) in high density pellet culture. This was verified by the demonstration that, after infection with Ad-GDF5, GDF5 was expressed at levels equivalent to those previously used for application of exogenous protein to pellet cultures. Further, the expression of chondrocyte markers was markedly induced, both at the mRNA and protein level, in the infected ADSCs. This is the first report describing the effects of GDF5 gene therapy on the chondrogenesis of ADSCs. The results demonstrated that adenovirus GDF-5 could augment chondrogenic potential of ADSCs and indicated that genetically modulated chondrogenic ADSCs could be an alternative cell resource for cartilage repair.


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