HIGH EFFICIENCY OF ADENO VIRAL GENE TRANSFER TO MATURE MYOFIBERS BY EX VIVO GENE TRANSFER BASED ON ADENO VIRUS HELPER CELLS

*Kimura, S; *Pruchnic, R; *Cao, B; +*Huard, J
+*Department of Orthopaedic Surgery, University of Pittsburgh, Pittsburgh, PA. 4151 Rangos Research Center, 3705 Fifth Ave., Pittsburgh, PA 15213-2582, 412-692-7807, Fax: 412-692-7095, jhuard+@pitt.edu

Introduction: Duchenne muscular dystrophy (DMD) is an X-linked recessive disease caused by the deficiency of dystrophin. Various techniques, i.e. cell transplantation, gene therapy, and myoblast-mediated ex vivo approach, have been used to restore the dystrophin gene in dystrophic muscle. The adenovirus displays advantageous features as a gene delivery vehicle to skeletal muscle: the ability to prepare high titer of the recombinant virus, the ability to infect a wide variety of cell types and tissue, the capability to infect quiescent, non-dividing cells such as myofibers and the ability to encode the full length dystrophin with the third generation adenovirus. However, the low efficiency of adenoviral transduction in mature myofibers remains an important hurdle that has to be overcome for the overall application of adenoviral gene therapy to skeletal muscle. The barriers that hinder the adenoviral penetration and transduction have recently been identified: the physical barrier imposed by the basal lamina, the lack of myoblast mediation within muscle development, and the abundance and availability of the adenoviral receptors in mature myofibers. Although many techniques to overcome these barriers are under investigation, the use of the myoblast mediated ex vivo gene transfer has been found capable to improve adenoviral transfer in adult muscle. Here, we have further developed the ex vivo gene transfer technique of adenovirus to skeletal muscle by using new adenovirus helper muscle cells, and demonstrated a significant improvement of gene transfer to mature myofibers.

Methods: The adenovirus used are replication-defective due to a deletion in the E1 gene (early region 1), eliminating viral proliferation and making them safer and effective for gene transfer. The production of these vectors requires expression of E1 genes in the producer cells to obtain titer high enough for injection. We have developed new helper cells by using: 1) a plasmid including the E1 gene (11% of early nucleotide sequence of Adenovirus type5), 2) the LacZ gene, and 3) the neomycin resistance gene (Fig. 1). The transfection was done using this plasmid and primary myoblasts from mdx mice (animal model for DMD) according to the standard protocol for lipofection. After screening by G418, new helper cells (E32) were obtained. E32 and 293 cells infected with adenovirus carrying the LacZ marker gene were injected into skeletal muscle of adult SCID (immunodeficient) and mdx mice. These mice were sacrificed at 5 days post-injection, and the injected muscle was removed and histochemically stained for LacZ and dystrophin. The expression of β-galactosidase was quantified by a LacZ assay (ONPG). The results were compared among the different groups, which included the ex vivo with E32 cells, 293 cells, myoblasts, and direct viral injection techniques. All experiments were approved by the authors’ institution, following the guidelines laid out by the USA Department of Health and Human Services.

Results: Colonies appeared and were picked up 10 days after screening by G418. The 100% LacZ positive cells were followed for these experiments. Figures 2 and 3 show that the number of transduced myofibers was significantly higher in the injected muscle by using the ex vivo approach (A, B, C) than the direct gene transfer of adenovirus (D). Moreover, the use of E32 (A) and 293 (B) cells in the ex vivo approach improved the efficiency of adenoviral gene transfer when compared to regular unmodified myoblasts (C). The level of gene transfer has been monitored using the β-galactosidase assay among the different groups: ex vivo gene transfer with 293 cells and E32 displayed the highest efficiency of adenoviral gene delivery to mature skeletal muscle, while direct viral injection yielded only a few positive myofibers. These experiments were then repeated in adult SCID mice to avoid immune rejection (data not shown). Many positive myofibers were detected in muscle injected with 293 and E32 cells. Several mature fibers that were scattered throughout the muscle were positive for LacZ (Figure 4). No positive fibers could be detected in muscle that was directly injected with adenovirus. The new transduction methods using 293 and E32 cells offered a better gene transfer than previous methods using primary myoblasts and direct injection.

Discussion: We showed high efficiency in gene transduction to skeletal muscle in adult mice using helper cells. This method is advantageous because 1) the transduction efficiency is high despite the use of low titers of adenovirus and 2) it allows transduction of adult myofibers. The mechanism by which adenovirus can transduce mature myofibers using the new ex vivo protocol based on helper cells remains unclear. The release of substances, such as protease, by the helper cells which may fenestrate the basal lamina and, consequently, allow adenoviral penetration and transduction in mature myofibers is being investigated. In fact, the basal lamina has been identified as a major barrier to transduction of mature myofibers. The development of a gene therapy protocol in which we inject helper cells into injured tissue to serve as a reservoir of secreting molecules as well as a factory to produce virus may improve the efficiency of gene therapy for many tissues of the musculoskeletal system.

Figure 1. Structure of the E1 of adenovirus type 5, LacZ, and neomycin resistance gene. This vector was used for myoblast transfection. Figure 2. LacZ staining of the injected muscle of adult mdx mice at 5 days post-injection. The new methods of E32 (A) and 293 cells (B) were compared with adenovirus mediated ex vivo approach (C) and direct injection (D). Figure 3. The level of β-galactosidase expression amongst the different groups. Figure 4. Transduction of mature myofibers with 293 cells at high magnification.

Acknowledgements: We would like to thank Marcelle Pellerin for technical assistance.

References: