THE USE OF MUSCLE-SPECIFIC PROMOTER TO IMPROVE ADENOVIRAL MEDIATED GENE TRANSFER TO SKELETAL MUSCLE

+ Department of Orthopaedic Surgery, 4151 Rangos Res Ctr, Children's Hospital of Pittsburgh, Pittsburgh, PA 15213. 412-692-7807, Fax: 412-692-7095, jhuard@pitt.edu

Introduction: Adenoviral transduction of myofibers still faces many problems including the immune rejection against the transduced cells. Transgenes constructed under a general promoter can also result in the transduction of nonmuscle cells. Any ectopic expression may be toxic and may also induce or strengthen a systemic immune response directed against the viral vector or the transgene itself (1). The safety and effectiveness of vector delivery could be improved by developing methods to ensure tissue-specific transgene expression. The muscle creatine kinase (MCK) gene is highly active in all striated muscles. MCK is the most abundant nonmitochondrial mRNA that is expressed in all skeletal muscle fiber types (2) and is also highly active in cardiac muscle (3,4). The MCK gene is not expressed in myoblasts, but becomes transcriptionally activated when myoblasts commit to terminal differentiation into myofibers (5,6). In this report, we tested the efficiency of gene transfer mediated by adenoviruses that encode for luciferase reporter gene under the control of cytomegalovirus (CMV) promoter (ADCMV) or MCK promoter (ADMCK). The efficiency of gene transfer, the long-term persistence of the transgene and finally the presence of immune response were monitored and compared among the different viral vectors.

Materials and methods: The policies and procedures of the animal laboratory are in accordance with those detailed by the USA Department of Health and Human Services. Animals and viruses: Immature (up to 7 days old) and mature (at least 8 weeks old) normal mice (strain C57BL/10J) and C57BL/10JScSnDmd-mdx<sup>+/−</sup> mice were used for the experiments. Adenoviruses used were with luciferase reporter gene under the CMV promoter or MCK promoter. Luciferase assay: Luciferase assay was done with the Luciferase Assay System (Promega, Madison, WI) according to the instructions of the manufacturer. Briefly, to produce a standard curve of light units vs. relative enzyme concentration, serial dilutions of luciferase (Promega) were made with 1 x Reporter Lysis Buffer. Muscles were weighed, homogenized and centrifuged. The supernatant was subjected to the Light emitting system for measurement of luciferase activity.

Transduction in adult mice: 10<sup>6</sup> pfu ADCMV and ADMCK were injected into hindlimb muscles of newborn mice. Mice were infected at 7, 14, 30, 60 days post-injection. Injected muscles were collected and checked for luciferase expression. Viral transduction in adult mice using <i>ex vivo</i> approach: Muscles were dissected and homogenized. The supernants were mixed with Luciferase Assay Reagent, and the light produced was read in a luminometer (Monolight 2010, Analytical Luminescence Laboratory, San Diego, CA). Luciferase activity was expressed as light units per mg protein.

Discussion: Adenoviral gene transfer to skeletal muscle was hindered by immune rejection. The previous strategy of constructing adenovirus was inserting the transgene under a general (viral) promoter. Transgene expression was high shortly after virus delivery, but the nonspecific expression of transgene triggered acute immune response, which lead to a rapid elimination of transgene. In this study, we found that both ADCMV and ADMCK can transduce immature muscle efficiently. ADMCK achieved better efficiency and persistence than ADCMV. Secondly, the same phenomenon was observed in adult regenerating muscle when the <i>ex vivo</i> approach was used. Transducing EP cells with ADMCK achieved better efficiency and persistence compared to using ADCMV. The immunostaining for CD4/CD8 showed that ADCMV triggered more immune response compared to ADMCK. We hypothesize that using muscle specific promoters in the construct of adenovirus leads to specific transgene expression in muscle and avoid transduction of antigen presenting cells (APC), therefore achieving good transduction and long term persistence.

Acknowledgements: Marcelle Pellerin (technician), James Cummins, Arvydas Usas, Cathy Wypychowski (secretary), Funding support: National Institute of Health (NIH PO1 AR 45926-01), Muscular Dystrophy Association (USA), Parent Project (USA)


48th Annual Meeting of the Orthopaedic Research Society
Poster No: 0308