Cell engineering for muscle gene therapy: Extemporaneous production of retroviral vector packaging macrophages using defective herpes simplex virus type 1 vectors harbouring gag, pol, env genes

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Abstract

Gene therapy as a treatment for neuromuscular diseases is an ever-developing concept based on the use of DNA as the therapeutic agent. In the search for appropriate strategies a bottleneck exists, however, concerning the targeting of vectors carrying the therapeutic gene, to all pathologic sites. These diseases are often characterised by multiple widespread lesions spread over a large area, rendering administration by local injection into tissues, clinically irrelevant. With this in mind, we have proposed that circulating cells (monocytes/macrophages), which home naturally to inflammatory lesions, characteristic of degenerating muscle, could be used as shuttles able to track down every damaged site, and deliver there a corrective gene. Our aim is to mobilise a corrective gene from these infiltrating monocyte-macrophages, into muscle cells, a process of *in situ* cell to cell gene transfer which could be accomplished using a retroviral vector, since the regeneration process involves the proliferation of muscle precursors before they fuse to form replacement fibres. For this, monocyte-macrophages must be engineered into 'packaging cells' containing both the replication deficient retrovirus carrying the gene of interest and an helper genome (gag-pol-env) needed for its assembly and secretion. Here, we have transduced a monocyte cell line using a defective murine Moloney leukemia retrovirus carrying the *LacZ* reporter gene. This provided us with a platform to investigate the possibility of gag-pol-env vector driven packaging of the defective retrovirus by macrophages. We show that an herpes simplex virus type I amplicon harbouring the Moloney gag, pol, env sequences is able to rescue the defective retrovirus vector from macrophages, allowing gene transfer into muscle precursor cells. After fusion, these cells gave rise to genetically modified myotubes *in vitro*.

Introduction

Typically, gene therapy for muscular dystrophies seeks to transfer a healthy or corrective gene into muscle fibres. This transfer has, so far, been achieved through injection of recombinant viral vectors (Quantin et al., 1992; Ragot et al., 1993; Vincent et al., 1993; Deconinck et al., 1996; Snyder et al., 1997), preparations of naked DNA (Wolff et al., 1990; Acsadi et al., 1991), or lethally processed murine packaging cells (Fassati et al., 1996, 1997), directly into the affected tissues. Delivery by local injection is, however, of limited

clinical use for muscle dystrophies where the sites to be treated are multiple, disseminated throughout the skeletal muscle as a whole, and often inaccessible. Indeed, specific widespread targeting currently represents a bottleneck in the search for appropriate strategies for the gene therapy for these diseases. Ideally, a targeting strategy needs to be sought which is adapted to disseminated lesions and so uses the systemic pathway, but nevertheless specifically limits the delivery of the therapeutic agent to the pathologic sites. To this end, we have proposed that circulating cells, such as monocyte-macrophages, which home

Figure 1. Schematic representation of gene delivery by homing macrophages at sites of muscle necrosis-regeneration. ① Muscle fibre necrosis leads to infiltration of blood borne macrophages (hatched cytoplasm). ② Surrounding satellite cells (grey cytoplasm) proliferate rendering possible a cell to cell gene transfer using retroviral vectors (●) secreted by 'packaging' macrophages. ③ Subsequent fusion of satellite cells results in genetically modified fibres.

naturally to inflammatory sites characteristic of degenerating muscle lesions, could be used as shuttles to track down every damaged area and deliver there a corrective gene (Parrish et al., 1996).

In muscular dystrophies, in particular Duchenne Muscular Dystrophy (DMD), the genetic defect results in the sporadic necrosis of muscle fibres, whose breakdown triggers a rapid infiltration of blood borne cells, particularly macrophages, into the tissue (Carlson, 1986; Orimo et al., 1991). Subsequently, for a period of time (Schultz and Lipton, 1980; Emery, 1993), a certain plasticity allows the compensation of this degenerative process by at least a partial regeneration, involving the activation and proliferation of satellite cells which then fuse to form new replacement muscle fibres. In fact, this transient proliferative phase offers the possibility of transferring a corrective gene into dividing satellite cells using a retroviral vector, and as has been shown (Fassati et al., 1996, 1997), the ensuing fusion of these cells can then generate genetically corrected replacement fibres. Our aim is

to use monocyte-macrophages to deliver such vectors, via the systemic pathway, to each and every site of necrosis-regeneration. This process of *in situ* cell to cell gene transfer, between macrophages and target muscle cells, would require that the former be engineered into a 'packaging cell' containing both the replication deficient retrovirus carrying the gene of interest and an helper genome (gag-pol-env) needed for its assembly and secretion (Figure 1). In this way, monocyte shuttles distributing the therapeutic retrovirus to all widespread sites of necrosis-regeneration, would allow the 'correction' of an ever increasing number of fibres as they regenerate. As a first step in this strategy, we have investigated, *in vitro*, the possibility of extemporaneously creating macrophage 'packaging cells', using an herpes simplex virus type I (HSV1) amplicon harbouring the Moloney gag-pol-env sequences to drive the mobilisation of a defective retrovirus (MoMuLV) carrying the *LacZ* reporter gene. We show here, that these engineered cells are able to transfer a gene into proliferating C2C12 muscle precursor cells, thus generating, after fusion, genetically modified myotubes.

Material and methods

Tissue culture

We used a monocyte-macrophage cell line (MØ9) obtained from the bone marrow of a transgenic mouse carrying a thermosensitive SV40 T-antigen under the control of the human vimentin promoter (Vicart et al., 1994). Culture was performed in bacteriological Petri dishes to limit cell adhesion to the substratum and thus maintain the cells in a monocyte-like morphology. Cells were cultivated in Rosslyn Park Memorial Institut (RPMI 1640) medium (Gibco) with 10% foetal calf serum (Serotec) and 10% 929 cell conditioned medium (which promotes growth of cells of the myeloid lineage). They were split every 4 days to give a cell concentration of about 2×10^5 cells mL^{-1} .

C2C12 muscle precursor cells were cultivated at low density in Dulbecco's Modified Eagle's Medium (DMEM) (Gibco) with 10% foetal calf serum. For fusion, cells were allowed to reach confluence and switched into DMEM with 10% horse serum (Serotec).

The GPE86 packaging fibroblast cell line was used to produce an ecotropic murine Moloney defective retrovirus carrying the reporter gene *LacZ* under the

control of the 5' LTR (MoLacZ). Cells were cultured in DMEM with 10% foetal calf serum and passaged at confluence every 4 days. Enriched supernatants were collected for the transduction of Mø9 monocyte-macrophages.

Immuno-histochemistry and ultrastructural analysis

For Mac1 immunochemistry, Mø9 cells were seeded into petri dishes. After fixation for 6 min in methanol at -20°C and washing in phosphate buffered saline (PBS), cells were incubated for 1 hr in PBS-T (PBS containing 0.3% triton X100) plus 10% normal goat serum (blocking buffer), followed by incubation overnight at 4°C with an anti-Mac1 rat monoclonal antibody (Serotec) diluted 1/50 in blocking buffer. Following washing in PBS-T, cells were incubated for 1 hr at room temperature with a biotinylated goat anti-rat antibody (Vector) diluted 1/200 in PBS-T. Antibodies were revealed using an ABC Elite peroxidase complex (Vector) followed by DAB (3,3'-diaminobenzidine) substrate according to manufacturer's procedure.

To reveal β -galactosidase activity, cells were first rinsed with PBS, then fixed for 1 hr in 2.5% glutaraldehyde (Aldrich), washed thoroughly in PBS, and incubated overnight at 4°C in a solution containing 2 mM 5-bromo-4-chloro-3-indolyl- β -D-galactopyranoside (X-gal Sigma), 5 mM potassium ferrocyanide, 5 mM potassium ferricyanide, and 2 mM MgCl_2 in PBS.

For electron microscopy, poorly adherent cells were gently resuspended, rinsed and pelleted for fixation for 1 hr in 2.5% glutaraldehyde in 0.1 M phosphate buffer pH7.4 at 4°C , followed by 0.6% glutaraldehyde in the same buffer overnight. Post-fixation was performed in 2% osmic acid in 0.1 M phosphate buffer for 1 hr at 4°C . Samples were dehydrated in graded alcohol and embedded in epon resin. Ultrathin sections (65 nm) were stained with lead citrate for 4 min. Sections were then observed using a Philips EM410 electron microscope (accelerating voltage 80 kV, objective aperture 20 μm).

Transduction procedures

For transduction of Mø9 cells by HSV1-LacZ recombinant vector, 10^6 cells were gently resuspended and centrifuged at 800g for 5 min. The pellet was recovered in 100 μL of transduction medium comprising DMEM with 1% foetal calf serum and containing

10^7 pfu HSV1-LacZ recombinant vector particles. After incubation for 1 hr and 30 min at 37°C , cells were rinsed and replated in 2 mL of complete medium for 24 hr before analysis for β -galactosidase activity.

Mø9-MoLac cells were created by transduction of Mø9 with a recombinant Moloney retrovirus (type LXS-N) (Miller and Rosman, 1989) consisting of the 5' and 3' LTR sequences encompassing packaging signal, LacZ gene under the control of the 5' LTR, and the neomycin resistance gene under the control of the SV40 promoter. The retrovirus was produced at high titre (10^6 particles mL^{-1}) by GPE86 (ecotropic) producer cells. For transduction, GPE86 conditioned supernatants were filtered through 0.45 μm cellulose acetate filtration unit (Millipore), diluted with an equal volume of Mø9-MoLacZ medium and transferred onto dividing Mø9-MoLacZ cells in the presence of 8 $\mu\text{g mL}^{-1}$ Polybrene (Sigma).

HSV1-GPE amplicon consists of a plasmid containing the HSV1 origin of replication and packaging signal, with Moloney 'gag-pol-env_{ecotrope}' sequences under the control of the CMV promoter (Savard et al., 1997). Amplicon vectors were generated using a defective HSV1 vector as helper virus, as previously described by Savard et al (Savard et al., 1997). For transduction of Mø9-MoLacZ cells by HSV1-GPE amplicon, 10^6 cells were pelleted and resuspended in 100 μL of DMEM containing 1% foetal calf serum plus 5 μL of the amplicon stock solution (2×10^7 helper pfu, 2×10^6 amplicon particles mL^{-1}). This gave 10^5 helper pfu, 10^4 amplicon particles, for 10^6 cells. After 1 hr and 30 min at 37°C , cells were rinsed, replated in 2 mL of complete medium, and the supernatant collected 48 hr later, to test for the presence of rescued retroviral particles.

Recovered supernatants were filtered through 0.45 μm cellulose acetate filtration units (millipore) and transferred onto dividing C2C12 cells in the presence of 8 $\mu\text{g mL}^{-1}$ of polybrene. Cultures were assessed for β -galactosidase activity at different time points.

Results and discussion

Ideally, in a therapeutic context, the use of macrophages in this way would require the recovery of their precursors (monocytes) from the blood compartment, their extemporaneous engineering into conditional packaging cells and their immediate reinfusion. Conceptually, the creation of a packaging cell

Figure 2. Viral vectors for monocytic-macrophage engineering. Recombinant defective provirus: 2 LTRs (Long Terminal Repeats), packaging signal (ψ^+), and a gene of interest under the control of an internal promoter (P). Carrying vector for provirus: adenoviral, herpes simplex virus or amplicon backbone, containing the provirus to be mobilised. Carrying vector for helper genome: adenoviral, herpes simplex virus, amplicon or lentiviral backbone, containing the sequences encoding 'gag-pol-env' from MoLV for mobilisation of the provirus.

can be reduced to a two-step procedure: i) introducing the proviral sequences into the cell, ii) conferring the functional ability to assemble and secrete the resulting retrovirus. Because circulating monocytes are essentially post-mitotic, introduction of both the proviral sequences to be mobilised, and the helper genome, would need to be accomplished using 'carrying' vectors compatible with somatic cells (Figure 2). This could be achieved using, for example, hybrid vectors comprising a 'carrying backbone' containing a complete recombinant defective murine provirus (with its two LTRs, packaging signal, and gene of interest), and the sequences encoding 'gag-pol-env' helper genes, respectively. As a first step, we chose to concentrate on the helper genome component. To simplify the experimental paradigm, a monocyte-macrophage cell line was used, whose high rate of proliferation permitted introduction of the provirus, harbouring the *LacZ* reporter gene, in a conventional manner. This provided us with a platform to investigate an HSV1-MLV 'gag-pol-env' hybrid vector as a tool to introduce into the macrophage the helper genome necessary to drive the mobilisation of a provirus.

Vectors for monocyte-macrophage engineering

We used an immortalised monocyte-macrophage cell line (MØ9) developed from the bone marrow of a transgenic mouse carrying a thermosensitive SV40 T-antigen under the control of the human vimentin promoter (Vicart et al., 1994). This cell line gives

rise to two distinct morphologies: one where the cells are flattened and adhere strongly to the substratum, the other where the cells are round due to their poor attachment. Although certain antigenic differences exist between the two, for example, the non adherent are positive for SER4 (sheep erythrocyte receptor) a marker for non-activated monocytes undergoing differentiation (Crocker and Gordon, 1989), whereas the adherent type expresses macrophage antigen Mac1 (Figure 3a), and were also able to rapidly phagocytose dyed latex beads (particle diameter $0.8 \mu\text{m}$). We focused our attention on the poorly adherent cells because their morphology and state of differentiation represent more closely that of circulating monocytes. Ultrastructural analysis confirmed that these poorly adherent cells displayed a multilobed chromatin dense nucleus, a vacuolated cytoplasm, and numerous digitations, characteristic of a mononuclear phagocyte lineage (Figure 3b). Thus, despite the fact that these cells are immortalised by SV40 T-antigen, which induces their proliferation providing an inexhaustible source, they maintain classical monocyte-macrophage characteristics. Furthermore, since the transgene is silenced *in vivo* (Vicart et al., 1994), these cells are non-tumorigenic and, when re-infused into mice, infiltrate inflammatory lesions as primary monocyte-macrophages (Parrish et al., 1996).

Figure 3. Characterisation of the Mø9 cell line. a) Immunostaining for Mac1 (Complement Receptor 3 specific to monocyte-macrophages). Positive cells were revealed with an anti-Mac1 rat monoclonal antibody followed by biotinylated second antibody, peroxidase complex and finally DAB substrate. Two distinct morphologies can be distinguished: flattened strongly adherent cells (black arrow heads) and round poorly adherent cells (white arrow heads). Two negative controls were performed: i) incubation of primary antibody with C2C12 (Mac1 negative cells), ii) omission of first antibody (anti-Mac1) on parallel control cultures of Mø9. Bar = 20 μm . b) Electron micrograph of a single cell of the poorly adherent type, displaying characteristic multilobed chromatin dense nucleus, vacuolated cytoplasm, and numerous digitations ($\times 7100$).

Figure 4. HSV1 vector mediated gene transfer into Mø9 cells. Histochemical analysis for β -galactosidase activity in Mø9 cells 24 hr after transduction with a defective HSV1 recombinant vector carrying the *LacZ* reporter gene under the control of the CMV promoter. 10^6 Mø9 cells were transduced in a 100 μl suspension containing 10^7 recombinant vector particles. Control non-transduced Mø9 cells were negative for β -galactosidase activity. Bar = 30 μm .

These cells would therefore seem to be a valid source of macrophages to investigate their engineering.

Several types of vectors have been shown to be able to transduce, efficiently, post-mitotic cells, namely, adenovirus associated virus (AAV) (Snyder et al., 1997a, b; Peel et al., 1997), lentivirus (Naldini et al., 1996), adenovirus (Ragot et al., 1993; Vincent et al., 1993; Le Gal la Salle et al., 1993; Bilang-Bleuel et al., 1997), and herpes virus type 1 (HSV1) (Lawrence et al., 1996; Weir et al., 1996; Erbacher et al., 1996; Fraefel et al., 1997). However, of these, neither AAV nor lentiviral vectors allow the insertion of a genome large enough to cover gag-pol-env helper sequences under an independent promoter, or a complete proviral sequence, and adenoviral vectors, although posing no restraints concerning the insert size, unfortunately appear to be inefficient in transducing undifferentiated macrophage precursors, such as monocytes (Haddada et al., 1993; Huang et al., 1995; Kristoffersen et al., 1997; Wickham et al., 1997). On the other hand, the reported ability of HSV1 vectors to infect human monocytes (Weir et al., 1996) led us to believe that they might be appropriate for the transduction in sus-

pension of the poorly adherent cell population of the MØ9 cell line. To this end, the ability of herpetic vectors to infect MØ9 cells suspension was verified using a defective HSV1 recombinant for *LacZ*. After transduction, cells were plated and examined 24 hr later for β -galactosidase activity (Figure 4). The level of transduction (25 to 30%) confirmed the effectiveness of vectors derived from HSV1, on these cells. However, the considerable toxicity observed at 48–72 hr post-infection imposed the choice of a less noxious HSV1 derivative to perform the vectorisation of the ‘gag-pol-env’ genes. Recombinant HSV1 vectors, although defective, nevertheless possess a large part of the viral genome in order to assure their production, and unfortunately, the resulting viral proteins render the vector considerably toxic for the host cells. Amplicon derivatives of these vectors, however, since they consist of a plasmid containing the HSV1 origin of replication and packaging sequences but no other viral sequences, theoretically avoid this problem (Spaete and Frenkel, 1982). Although their need for a helper virus providing trans-acting functions for amplification means that preparations of amplicon particles are contaminated to some extent by helper viruses, despite this contamination, amplicon preparations are nonetheless considerably less toxic than their recombinant vector counterparts, especially when used at low MOI (Lowenstein et al., 1994). We chose, therefore, to use this form of the vector to vehicle the ‘gag-pol-env’ genome.

HSV1 ‘gag-pol-env’ amplicon driven extemporaneous packaging

The MØ9 cell line, due its high rate of proliferation was easily transduced with a defective retroviral vector (Mo*LacZ*) in a classical way. This allowed us to obtain a population of monocyte-macrophages of which greater than 70% were carrying the provirus to be mobilised (Figure 5a), thus providing a platform to investigate the possibility of ‘gag-pol-env carrier’ vector driven packaging by macrophages. This population of MØ9-Mo*LacZ* cells was subsequently transduced with HSV1-GPE amplicon (Savard et al., 1997), in which the ecotropic form of the env gene confers a murine specific tropism on the resulting retroviruses. Supernatants were conditioned for 48 hr after transduction and then transferred onto dividing C2C12 mouse muscle precursor cells. When C2C12 cells were examined 48 hr later for β -galactosidase activity (Figure 5b), several foci of positive cells

were observed, and when these cells were allowed to continue in culture, their fusion resulted in genetically modified myotubes (Figure 5c). Although it is true that *lacZ* retroviral vectors can theoretically be rescued by contaminating wild type corresponding virus, in our experiments this was not the case since pol-env’ amplicon, did not transduce C2C12 with the *LacZ* retrovirus. Our data, therefore demonstrate the ability of the HSV1 ‘gag-pol-env’ amplicon to confer extemporaneously the packaging function on monocyte-macrophages and thus to rescue defective *LacZ* retrovirus.

To estimate the production efficiency, the number of ‘hits’ per culture were counted, resulting in approximately 30 of 4×10^4 C2C12 cells. This apparently low level of gene transfer, rather than representing inefficient packaging by the macrophages, is in fact probably due to artifactual experimental restraints. First, in these experiments, the MØ9-Mo*LacZ* cells were transduced with HSV1-GPE amplicon corresponding to a multiplicity of infection (MOI) of 0.1 for the helper particles (above which toxicity was too important), which imposed a maximum MOI of 0.01 for the amplicon of interest. By definition, therefore, no more than 1% of the 10^6 MØ9-Mo*LacZ* cells could be transduced by the amplicon. Thus, it was possible to create a maximum of only 10^4 producers out of 10^6 cells. Second, due to this low MOI, only a small minority of monocyte/macrophages express the env protein at the surface, and thus, on the vast majority of cells, the receptor remains available for infection by retrovirus emerging from a neighbouring ‘packaging macrophage’. This phenomenon may thus result in a ‘mopping up’ of part of the rescued retroviruses from the supernatant, thus reducing its conditioning, an argument supported by the fact that higher titres are obtained with human producer cells, which express an amphotropic env retrovirus receptor not compatible with the ecotropic form of this virus (Savard et al., 1997). Given these two constraints then, the very few macrophages actually having received the HSV1-GPE amplicon, and therefore able to rescue the retrovirus, may well be doing so with great efficiency. The macrophage would therefore seem to be an appropriate cell type for this function, and the HSV1 amplicon a suitable tool to vehicle the required sequences.

In order to now transfer this technology to *in vivo* experimental paradigms, we need to improve the number of macrophages on which this packaging function can be conferred. Exposing the macrophage popula-

Figure 5. Gene transfer from extemporaneously engineered Mø9 monocyte-macrophage 'packaging' cells, into C2C12 muscle precursors. Histochemical analysis for β -galactosidase activity: a) Engineered monocyte-macrophages. 10^6 Mø9 cells were initially transduced with defective retrovirus recombinant for *LacZ* (non-transduced Mø9 cells were negative for β -galactosidase activity). Subsequently, these Mø9-Mo*LacZ* cells were transduced in suspension with 10^4 particles of HSV1 amplicon carrying the Moloney 'gag-pol-env' sequences, to extemporaneously confer packaging capacity on the macrophages. Bar = 50 μ m. b) Mobilisation of *LacZ* retroviral vector from Mø9-Mo*LacZ* to dividing C2C12 muscle precursor cells. C2C12 cells transduced with supernatant from culture (a) (conditioned for 48 hr). Negative controls were performed where the supernatant of Mø9 cells transduced with *lacZ* retrovirus but not with amplicon 'gag-pol-env' vector was transferred on C2C12 cells. No β -galactosidase activity was ever detected in these cells. c) Resulting genetically modified myotubes 10 days later.

tion to a greater number of amplicon particles would theoretically solve the problem. However, as things stand, this is difficult due to the limiting level of toxicity of vector preparations. Nonetheless, this toxicity coming, not from the amplicon itself, but from contamination of the preparation by helper vectors (as discussed earlier), it should be possible to overcome this barrier by improving vector preparation techniques, to produce amplicon batches devoid of contaminants (Fraefel et al., 1996; Jacoby et al., 1997).

Conclusion

To overcome the problem of targeting a therapeutic gene to all widespread sites of lesion in diseases such as muscular dystrophy, we have proposed a strategy using circulating cells (monocytes-macrophages), which home naturally to these sites, as shuttles able to deliver, via the systemic pathway, an appropriate vector carrying the gene (Parrish et al., 1996). With this in mind, as a first step, we have attempted here to determine the possibility of using macrophages as retrovirus 'packaging cells' able to transfer a gene into muscle precursor cells during *in vitro* myogenesis. We have demonstrated the ability of monocyte-

macrophages to perform this function, as well as the feasibility of accomplishing the engineering required to introduce the 'packaging' sequences extemporaneously, using an HSV1-amplicon backbone. Technical improvements in production of the engineering vectors are now required, however, before this approach can be evaluated *in vivo*.

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References

- Acsadi G, Dickson G, Love DR, Jani A, Walsh FS, Gurusinge A, Wolff JA and Davies KE (1991) Human dystrophin expression in mdx mice after intramuscular injection of DNA constructs. *Nature* 352: 815–818.
- Bilang-Bleuel A, Revah F, Colin P, Locquet I, Robert JJ, Mallet J and Horellou P (1997). Intrastriatal injection of an adenoviral vector expressing glial-cell-line-derived neurotrophic factor

- prevents dopaminergic neuron degeneration and behavioral impairment in a rat model of Parkinson disease. *Proc Natl Acad Sci USA* 94(16): 8818–8823.
- Carlson BM (1986) Regeneration of entire skeletal muscle. *Federation Proceedings* 45(5): 1456–1460.
- Crocker PR and Gordon SJ (1989) Mouse macrophage hemagglutinin (sheep erythrocyte receptor) with specificity for sialylated glycoconjugates characterized by a monoclonal antibody. *Exp Med* 169(4): 1332–1346.
- Deconinck N, Ragot T, Marechal G, Perricaudet M and Gillis JM (1996) Functional protection of dystrophic mouse (mdx) muscles after adenovirus-mediated transfer of a dystrophin minigene. *Proc Natl Acad Sci USA* 93(8): 3570–3574.
- Emery A, *Duchenne Muscular Dystrophy*, Oxford University Press, 1993.
- Erbacher P, Bousser MT, Raimond J, Monsigny M, Midoux P and Roche AC (1996) Gene transfer by DNA/glycosylated polylysine complexes into human blood monocyte-derived macrophages. *Hum Gene Ther* 7(6): 721–729.
- Fassati A, Wells DJ, Walsh FS and Dickson G (1996) Transplantation of retroviral producer cells for *in vivo* gene transfer into mouse skeletal muscle. *H Gene Therapy* 7(5): 595–602.
- Fassati A, Wells DJ, Sgro Serpente PA, Walsh FS, Brown SC, Strong PN and Dickson G (1997) Genetic correction of dystrophin deficiency and skeletal muscle remodeling in adult MDX mouse via transplantation of retroviral producer cells. *J Clin Invest* 100(3): 620–628.
- Fraefel C, Song S, Lim F, Lang P, Yu L, Wang Y, Wild P and Geller AI (1996) Helper virus-free transfer of herpes simplex virus type 1 plasmid vectors into neural cells. *J Virol* 70(10): 7190–7197.
- Haddada H, Lopez M, Martinache C, Ragot T, Abina MA and Perricaudet M (1993) Efficient adenovirus-mediated gene transfer into human blood monocyte-derived macrophages. *Biochem Biophys Res Commun* 195(3): 1174–1183.
- Huang S, Endo RI and Nemerow GR (1995) Upregulation of integrins alpha v beta 3 and alpha v beta 5 on human monocytes and T lymphocytes facilitates adenovirus-mediated gene delivery. *J Virol* 69(4): 2257–2263.
- Jacoby DR, Fraefel C and Breakefield XO (1997) Hybrid vectors: a new generation of virus-based vectors designed to control the cellular fate of delivered genes. *Gene Therapy* 4(12): 1281–1283.
- Kristoffersen AK, Sindre H, Mandi Y, Rollag H and Degre M (1997) Effect of adenovirus 2 on cellular gene activation in blood-derived monocytes and macrophages. *APMIS* 105(5): 402–409.
- Lawrence MS, Ho DY, Sun GH, Steinberg GK and Sapolsky RM (1996) Overexpression of Bcl-2 with herpes simplex virus vectors protects CNS neurons against neurological insults *in vitro* and *in vivo*. *J Neurosci* 16(2): 486–496.
- Le Gal La Salle G, Robert JJ, Berrard S, Ridoux V, Stratford-Perricaudet LD, Perricaudet M and Mallet J (1993) An adenovirus vector for gene transfer into neurons and glia in the brain. *Science* 259(5097): 988–990.
- Lowenstein PR, Fournel S, Bain D, Tomasec P, Clissold P, Castro MG and Epstein A (1994) Herpes simplex virus 1 (HSV-1) helper co-infection affects the distribution of an amplicon encoded protein in glia. *Neuroreport* 5(13): 1625–1630.
- Miller AD and Rosman GJ (1989) Improved retroviral vectors for gene transfer and expression. *BioTechniques* 7(9): 980–982.
- Naldini L, Blomer U, Gallay P, Ory D, Mulligan R, Gage FH, Verma IM and Trono D (1996) *In vivo* gene delivery and stable transduction of nondividing cells by a lentiviral vector. *Science* 272(5025): 263–267.
- Orimo S, Hiymuta E, Arahata K and Sugita H (1991) Analysis of inflammatory cells and complement C3 in bupivacaine-induced myonecrosis. *Muscle and Nerve* 14(6): 515–520.
- Parrish EP, Cifuentes-Diaz C, Li ZL, Vicart P, Paulin D, Dreyfus PA, Peschanski M, Harris AJ and Garcia L (1996) Targeting widespread sites of damage in dystrophic muscle: engrafted macrophages as potential shuttles. *Gene Therapy* 3(1): 13–20.
- Peel AL, Zolotukhin S, Schrimsher GW, Muzyczka N and Reier PJ (1997) Efficient transduction of green fluorescent protein in spinal cord neurons using adeno-associated virus vectors containing cell type-specific promoters. *Gene Therapy* 4(1): 16–24.
- Quantin B, Perricaudet LD, Tajbakhsh S and Mandel JL (1992) Adenovirus as an expression vector in muscle cells *in vivo*. *Proc Natl Acad Sci USA* 89(7): 2581–2584.
- Ragot T, Vincent N, Chafey P, Vigne E, Gilgenkrantz H, Couton D, Cartaud J, Briand P, Kaplan JC, Perricaudet M. et al. (1993) Efficient adenovirus-mediated transfer of a human minidystrophin gene to skeletal muscle of mdx mice. *Nature* 361(60413): 647–650.
- Savard N, Cosset FL and Epstein AL (1997) Defective herpes simplex virus type 1 vectors harboring gag, pol, and env genes can be used to rescue defective retrovirus vectors. *J Virol* 71(5): 4111–4117.
- Schultz E and Lipton PH (1980) *Mech Aging Dev* 13: 105–112.
- Smith MJ and Koch GLE (1987) Differential expression of murine macrophage surface glycoprotein antigens in intracellular membranes. *J Cell Sci* 87(Pt1): 113–119.
- Snyder RO, Miao CH, Patijn GA, Spratt SK, Danos O, Nagy D, Gown AM, Winther B, Meuse L, Cohen LK, Thompson AR and Kay MA (1997) Persistent and therapeutic concentrations of human factor IX in mice after hepatic gene transfer of recombinant AAV vectors. *Nat Genet* 16(3): 270–276.
- Snyder RO, Spratt SK, Lagarde C, Bohl D, Kaspar B, Sloan B, Cohen LK and Danos O (1997) Efficient and stable adeno-associated virus-mediated transduction in the skeletal muscle of adult immunocompetent mice. *Hum Gene Ther* 8(16): 1891–1900.
- Spaete RR and Frenkel N (1982) The herpes simplex virus amplicon: a new eucaryotic defective-virus cloning-amplifying vector. *Cell* 30(1): 295–304.
- Vicart P, Schwartz B, Vandewalle A, Bens M, Delouis C, Panthier JJ, Pournin S, Babinet C and Paulin D (1994) Immortalization of multiple cell types from transgenic mice using a transgene containing the vimentin promoter and a conditional oncogene. *Exp Cell Res* 214(1): 35–45.
- Weir JP, Dacquel EJ and Aronovitz J (1996) Herpesvirus vector-mediated gene delivery to human monocytes. *Hum Gene Ther* 7(11): 1331–1338.
- Vincent N, Ragot T, Gilgenkrantz H, Couton D, Chafey P, Gregoire A, Briand P, Kaplan JC, Kahn A and Perricaudet M (1993) Long-term correction of mouse dystrophic degeneration by adenovirus-mediated transfer of a minidystrophin gene. *Nature Genet* 5(2): 130–134.
- Wickham TJ, Tzeng E, Shears LL 2nd, Roelvink PW, Li Y, Lee GM, Brough DE, Lizonova A and Kovesdi I (1997) Increased *in vitro* and *in vivo* gene transfer by adenovirus vectors containing chimeric fiber proteins. *J Virol* 71(11): 8221–8229.
- Wolff JA, Malone RW, Williams P, Chong W, Acsadi G, Jani A and Felgner PL (1990) Direct gene transfer into mouse muscle *in vivo*. *Science* 245(4949): 1465–1468.

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