Technical Note

K2 Transfection System increases the efficiency of adenoviral transduction of murine mesenchymal stromal cells

Madalina Dumitrescu, Ana Maria Vacaru, Violeta Georgeta Trusca,
Ioana Madalina Fenyo, Radu Ionita and Anca Violeta Gafencu

Gene Regulation and Molecular Therapies laboratory, Institute of Cellular Biology and Pathology "Nicolae Simionescu", 8 B.P. Hasdeu Str., Bucharest, Romania;

Adenoviral vectors are important vehicles for delivering therapeutic genes into mammalian cells. However, the yield of the adenoviral transduction of murine mesenchymal stromal cells (MSC) is low. High doses required to produce effective gene transfer cause unwanted cellular toxicity. To improve the adenoviral transduction efficiency and the transgene expression, minimizing adverse reactions, we have searched for molecules (polycationic compounds or lipids) able to enhance adenovirus adsorption on the cell surface. From the variety of agents checked, K2 Transfection System (K2TS) was the most efficient in increasing the expression of the transgene (GFP in our case), as we recently described [1].

Adenovirus Packaging, Purification, and Titration. The adenovirus was obtained and titrated as we previously described in detail [2,3]. Briefly, pAdTrack adenoviral vector containing DNA encoding GFP under CMV promoter (plasmid #16405, Addgene) linearized with PmeI was used to transform AdEasier-1 cells (a gift from Bert Vogelstein #16399, Addgene). After selection, the recombinant DNA was amplified in DH5α cells, purified, and used for the transfection of AD293 cells, using the K2 Transfection System (K2TS, Biontex Laboratories). The adenovirus was further amplified in AD293 cells and then the virus was purified by ultracentrifugation at 210.000 g, (35,000 rpm) on a discontinuous CsCl gradient (1.2 g/L and 1.4 g/L), for 18 h at 4 °C. To determine the titer, AD293 cells seeded in 12-wells plates were transduced with adenoviral dilutions ranging between $1/10^4$ and $1/10^7$. GFP-expressing cells were determined by flow cytometry. The samples with 5–20% GFP-positive cells from the total population were taken into account for the calculation of viral titer using the following formula: Titer (TU/mL) = D × F/100 × C/V, where D is the dilution factor, F is the percentage of positive cells/100, C is the counted cells/well, and V is the volume of viral inoculum.

Adenoviral Transduction of MSC in the Presence of Various Potential Boosters. A total of 1×10^5 MSC were transduced with 2.5×10^7 adenoviral particles (corresponding to 250 TU/cell) alone (AdV) or in complexes with some polymers or cationic lipids, prepared as follows: (i) AdV + K2: 90 min before transduction, MSC were incubated with 10 µL K2 Multiplier (K2M); in the meantime, 2.5×10^7 adenoviral particles were diluted in 65 µL DMEM. This viral dilution was mixed with 5 µL K2 transfection reagent (K2TR) diluted in 65 µL DMEM. The mixture was incubated for 20 min at room temperature and then was added dropwise to the MSC; (ii) AdV + Lipofectamine: a mixture containing 2.5×10^7 adenoviral particles and 10 µL P3000 in 125 µL Opti-MEM was added over a dilution of 7.5 µL Lipofectamine Reagent in 125 µL Opti-MEM; the final mix was incubated for 20 min at RT and then was added dropwise to the MSC; (iii) AdV + Polybrene: 2.5×10^7 adenoviral particles were added in 250 µL DMEM containing 2.5 µL solution 10 mg/mL polybrene; the mixture was added dropwise to MSC; (iv) AdV + Cholesterol: 2.5 µL solution containing 0.4 mg/mL cholesterol (in ethanol) were mixed with 2.5×10^7 adenoviral particles diluted in 250 µL DMEM and incubated for 20 min at RT; then the mixture was added dropwise to the MSC; (v) AdV + Poly-L-Lysine: 2.5 µl solution containing 0.1 mg/mL poly-L-Lysine were mixed with 2.5×10^7 adenoviral particles in 250 µL DMEM, and then the mixture was incubated with MSC in 1 mL serum free medium for 90 min; after this, 100

µL FBS was added to the cells; (vi) AdV + TransFast: 21 µL TransFast in 100 µL DMEM were mixed with 2.5 × 10^7 adenoviral particles and incubated for 15 min at RT, then were added to the MSC; (vii) AdV + Viromer Red: 540 µL Viromer Buffer with 2.5 × 10^7 adenoviral particles were mixed with 2.5 µL Viromer Red in 57.6 µL Viromer Buffer and incubated 15 min at RT, then the mixture was added to the cells. Transfection of MSC with pAdTrack-CMV using K2TS was performed as followed: MSC were incubated with 10 µL K2M for 90 min; in parallel, 2.4 µg pAdTrack-CMV diluted in 65 µL DMEM were mixed with 5 µl K2 transfection reagent diluted in 65 µL DMEM and incubated 20 min at RT; then, the mixture was added dropwise to the MSC. Eighteen hours later, the medium was replaced and the cells were analyzed 48 h post-transduction. The presence of GFP was evaluated by fluorescence microscopy at 20x (Axio Vert A.1, Carl Zeiss Jena GmbH), or quantified by flow cytometry using CytoFLEX Flow Cytometer (Beckman Coulter Life Sciences).

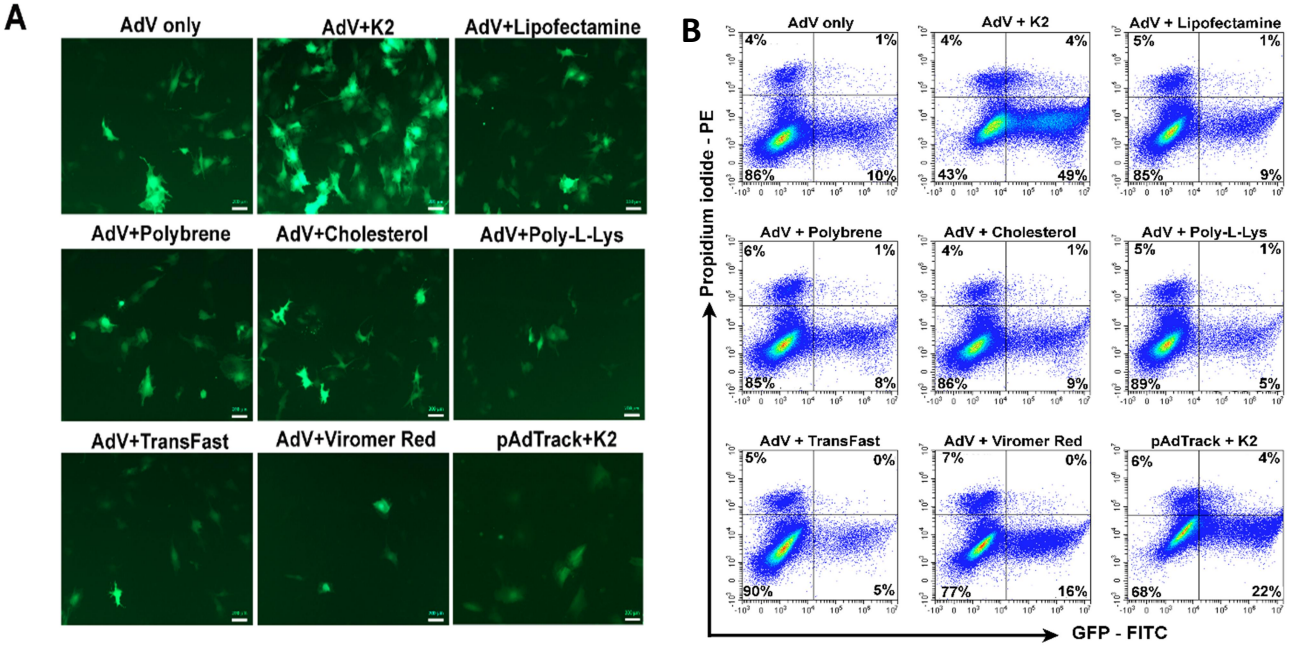

Figure 1. Adenoviral transduction of murine mesenchymal stromal cells (MSC) in the presence of different potential transduction boosters. To induce GFP expression, MSC were incubated with 250 TU/cell adenovirus alone (AdV only) or in the presence of the K2 Transfection System (K2TS) (AdV + K2), Lipofectamine 3000 (AdV + Lipofectamine), 10 µg/mL Polybrene (AdV + Polybrene), 2 µg/mL free cholesterol (AdV + Cholesterol), 1 µg/mL poly-L-Lysine (AdV + Poly-L-Lys), TransFast (AdV + TransFast), or Viromer Red (AdV + ViromerRed). In parallel, MSC were transfected with pAdTrack-CMV using K2TS (pAdTrack + K2). After 48 h, the GFP expression was observed by fluorescence microscopy (**A**), and the number of the GFP-expressing cells was determined by flow cytometry (**B**). The percentage of GFP-positive cells and the dead cells colored with propidium iodide (PI) was determined by flow cytometry. As is revealed, the K2TS is the most efficient reagent for boosting the adenoviral transduction of MSC. Bars, 20µm

Optimization of the K2TS Components for MSC Adenoviral Transduction. The doses of both K2TS components, K2TR and K2M were optimized. (i) To optimize K2TR concentration, MSC were incubated for 90 min with 10 µL/mL K2M in the culture medium. In parallel, 2.5 × 10^7 adenoviral particles diluted in 65 µL DMEM were mixed with increasing doses of K2TR (0.5–10 µL) diluted in 65 µL DMEM and incubated for 20 min at room temperature. (ii) To optimize K2M concentration, MSC were incubated with 1–50 µL K2M for 90 min. The complexes of the adenoviral particles with 5 µL K2TR were done as described above. In both cases, the mixture was added drop by drop to the MSC. After 18 h the medium was changed and the GFP expressing cells were analyzed 48 h after transduction.

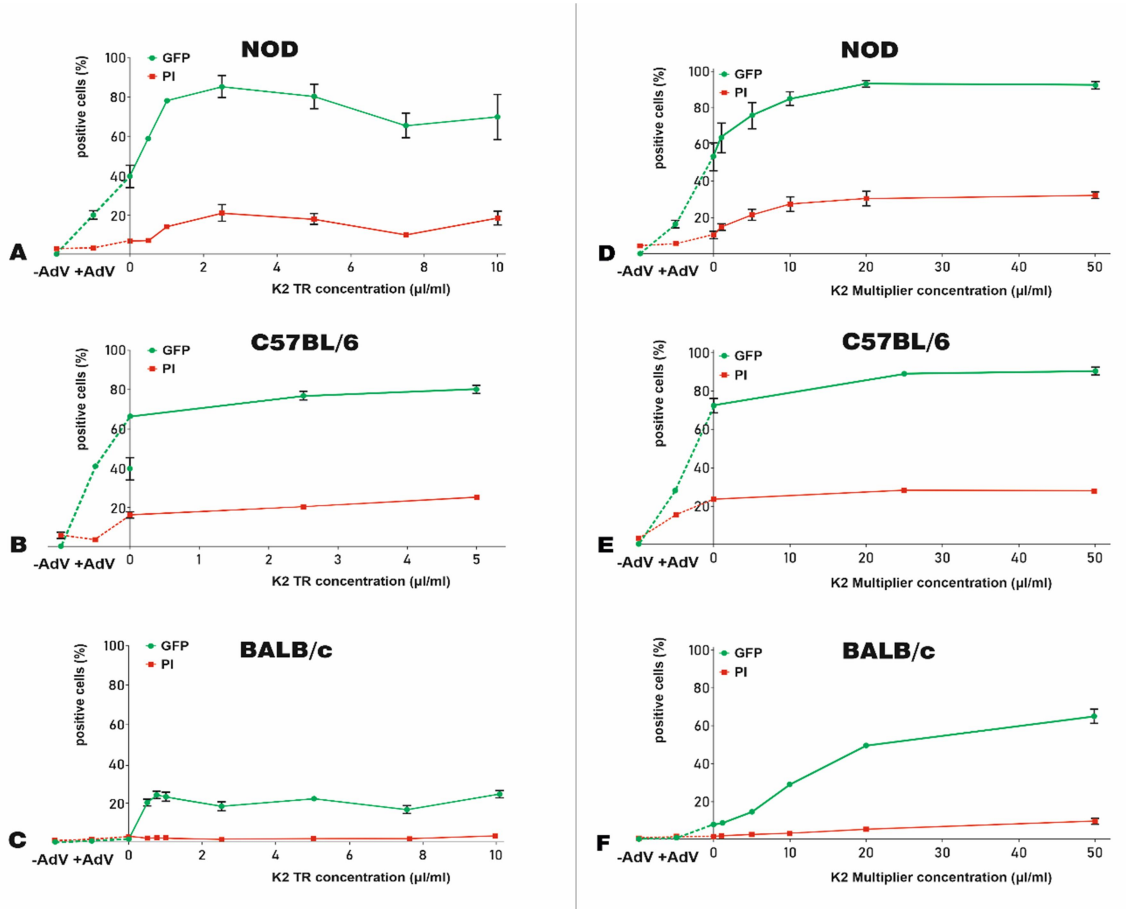

Figure 2. K2 Transfection System components optimization for the adenoviral transduction of murine MSC. The efficacy of K2TR to increase the yield of the adenoviral transduction of MSC derived from NOD (**A, D**), C57BL/6 (**B, E**), and BALB/c (**C, F**) mice were determined as % of GFP-positive cells (green lines) 48 h after transduction, by flow cytometry. The cell death was determined by PI incorporation and expressed as % of PI-positive cells (red lines). On the left side of each graph, the GFP-positive cells and the cell death for the untransduced cells (-AdV) and for the cells transduced with 250 TU/cell adenovirus alone (+AdV) were illustrated, linked by a dotted line. (**A-C)** Adenoviral particles were complexed with various K2TR doses. MSC were incubated with 10 µL/mL K2 Multiplier (K2M) and then the complexes of adenovirus–K2TR were added to the cells. (**D-F**) To optimize the concentration of K2M needed for transduction, MSC were incubated with increasing K2M concentrations (up to 50 µL/mL) for 90 min. Then, the adenovirus–K2TR complexes (5 µL/mL K2TR and 250 TU/cell adenoviral particle) were added to the cells.

Our data showed that MSC transduced in the presence of K2TS preserved their cellular markers (Sca-1 and CD73), their potential to differentiate into the adipogenic and osteogenic lineages, and their immunosuppressive effect (inhibiting proliferation of both CD4+ and CD8+ T cells).

These data better emphasize the importance of the transduction boosting by K2TS, as it allows the use of lower adenoviral doses to attain a good expression of the transgene.

In conclusion, K2TS proved to enhance the adenoviral transduction of MSC inducing an increased transgene expression, without affecting their intrinsic characteristics. This highly efficient method saves a lot of work, time, and money otherwise needed to obtain huge amounts of adenoviral particles for MSC transduction in the absence of a K2TS booster.

1. Dumitrescu, M.; Vacaru, A.M.; Trusca, V.G.; Fenyo, I.M.; Ionita, R.; Gafencu, A.V. K2 Transfection System Boosts the Adenoviral Transduction of Murine Mesenchymal Stromal Cells. *Int J Mol Sci* **2021**, *22*, doi:10.3390/ijms22020598.
2. Dumitrescu, M.; Trusca, V.G.; Fenyo, I.M.; Gafencu, A.V. An Efficient Method for Adenovirus Production. *JoVE* doi:10.3791/61691, e61691, doi:doi:10.3791/61691.
3. Dumitrescu, M.; Trusca, V.G.; Savu, L.; Stancu, I.G.; Ratiu, A.C.; Simionescu, M.; Gafencu, A.V. Adenovirus-Mediated FasL Minigene Transfer Endows Transduced Cells with Killer Potential. *Int J Mol Sci* **2020**, *21*, doi:10.3390/ijms21176011.