Use of Recombinant AdSV40-βGal Adenoviral Construction for Monitoring of Transplanted Cells

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Original recombinant adenoviral construction carrying *E. coli* β-galactosidase LacZ gene designed by the authors is convenient for labeling and monitoring of bone marrow mesenchymal (stromal) progenitor cells and myocardial and skin fetal cells transplanted in damaged rat tissues (in the perinecrotic zone of the myocardium and onto burnt skin surface) for their reparation. This genetic construction after pre-inactivation of endogenous β-galactosidase allows to detect transplanted cells in the foci of injury; positive effects of transplantation on tissue reparation processes can be attributed to the presence of transplanted cells.

Key Words: *AdSV40-βGal; stromal progenitor mesenchymal cells; bone marrow; β-galactosidase*

The potentialities of cell therapy are now actively studied in model experiments on animals. The available methods for cell delivery to organs cannot always ensure their penetration in the studied zone. This necessitates cell monitoring, detection of cell distribution in the organ, and confirmation of their viability after transplantation to the host.

Different methods of cell monitoring are known. One of the most often used is based on the detection of the expression of marker genes inserted into cells. The products of marker gene should not be toxic for live cells, impair its viability and morphology, or cause immune response of the host. The construction for introduction of the marker gene should be effectively inserted in cells and the product of gene expression should be easily identified by available methods.

Among the most popular markers are *E. coli* β-galactosidase [2-4,6-8,10-15,17,18] and *Aequorea victoria* "green protein" [5,9,14,16]. There are other markers, for example, red fluorescent protein derived from *Dismosoma sp*. Phosphatase gene is also a useful reporter gene [10].

We used an original recombinant adenoviral vector (AdSV40-βGal) with *E. coli* LacZ gene encoding β-galactosidase for monitoring of the delivery of stem (progenitor) and fetal cells to a certain area and viability of these cells and functioning at different periods after transplantation.

MATERIALS AND METHODS

Original recombinant AdSV40-βGal adenoviral construction was created in cell strain 293 by homologous recombination between linearized pAd-βGal plasmid DNA (Sileks M Firm) and a large Ad5 fragment, obtained by restriction of this adenovirus by C1a1. Plasmid pAd-βGal contained a cassette consisting of a nucleotide fragment (454 n. p.) of the 5'-terminal inverted adenoviral repeat, LacZ gene under SV40 promotor, and part of the adenovirus (3328-6241 n. p.) needed for homologous recombination. DNA transfection into cells was carried out by the calcium phosphate method. Viral plaques obtained after homologous recombination were collected and analyzed for

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their capacity to express LacZ gene. The recombinant adenovirus was produced in cells 293 (carrying genes necessary for multiplication of deficient AdSV40-βGal adenovirus), concentrated in CsCl density gradient, and resultant construction was confirmed by restriction mapping. The recombinant virus was titered, reproduced, if needed, in cell culture 293, and stored at -70°C.

Cells for infection with recombinant virus were cultured in DMEM/F-12 or Iscov's medium (Sigma) with 10% FBS (HyClone) and 40 μg/ml gentamicin. The infection was carried out overnight (12 h) in a medium with 0.5% serum. After incubation at 37°C in a CO_2 incubator the medium was removed from cell culture, the cells were washed twice with Hanks' solution, and growth medium was added. On day 5 postinfection the cells were fixed, heated for 40 min at 50°C for inactivation of endogenous β-galactosidase, and stained for bacterial β-galactosidase activity using X-Gal substrate [2,7]. Stained cells were washed in normal saline and the cells with characteristic blue staining were detected under a microscope.

In order to obtain bone marrow stromal stem cells and fetal cells labeled with AdSV40-βGal, they were infected by incubation with titered suspension containing AdSV40-βGal 12 h before transplantation into damaged organs. Directly before transplantation labeled cell culture was washed 3 times in Hanks' solution, the cells were removed by standard treatment with trypsin-versain solution, precipitated twice by centrifugation at 1500 rpm at ambient temperature, and resuspended in Hanks' solution. Viable cells were counted using trypan blue staining. Cultures with 95% viability were used for transplantation.

The survival of transplanted cells was studied on Wistar rats with experimental myocardial infarction induced by cryodestruction and with deep thermal burns of the skin.

On day 2 after burn was inflicted, the necrotic crust on the wound surface was resected and suspension of fetal fibroblasts or fibroblast-like bone marrow mesenchymal stem cells (MSC) in a dose of 2×10^4 cells per cm^2 of burn surface was applied onto the wound. The regeneration efficiency was evaluated by the rate of wound surface shrinkage using the planimetric method.

On day 7 after myocardial infarction, 2×10^6 labeled fetal cardiomyocytes or cardiomyocyte-like bone marrow MSC were injected into the perinecrotic zone of the myocardium. The results of cell therapy were evaluated by changes in the myocardial contractile function of isolated heart on a testing unit by Neely's

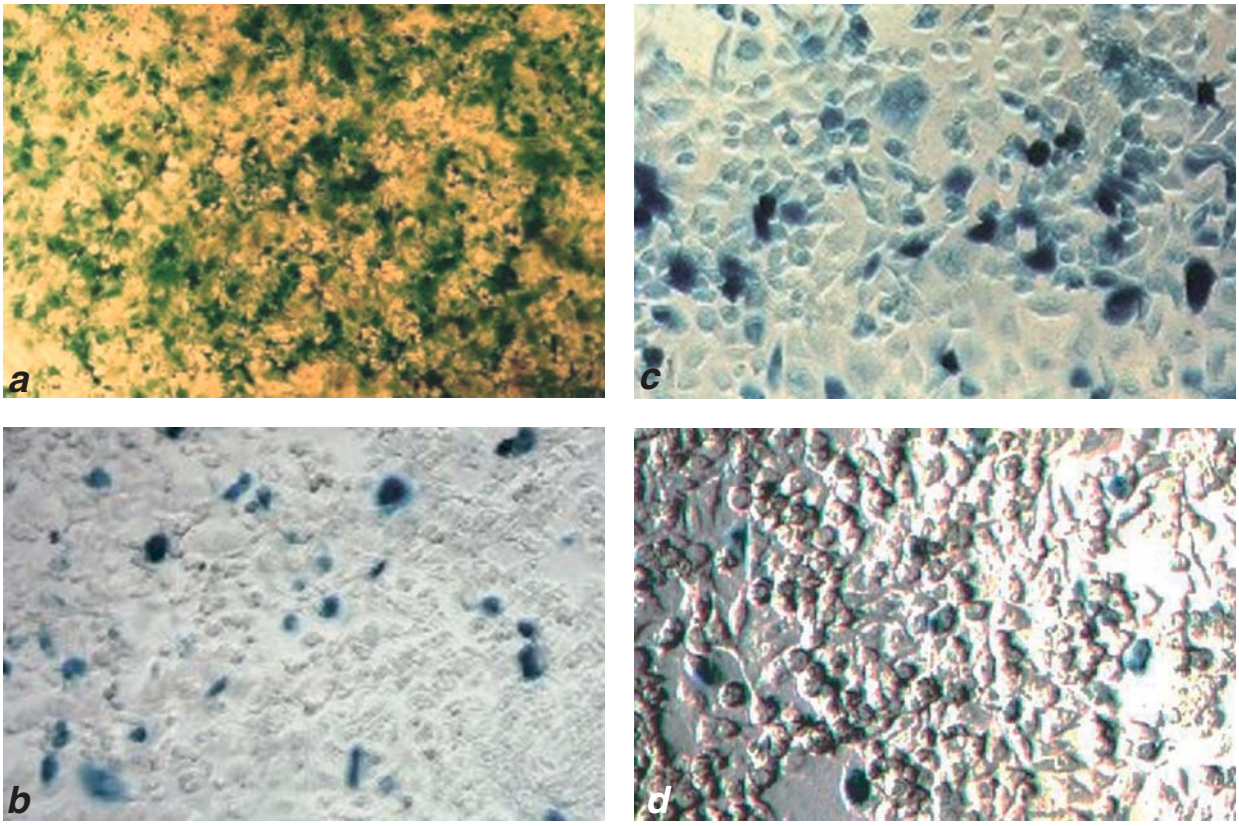

Fig. 1. Cultures of HepG2 (*a*; ×50), HeLa (*b*; ×200), MDCK (*c*; ×200), and Vero (*d*; ×200) cells labeled with AdSV40-βGal and stained for β-galactosidase activity on day 5 postinfection.

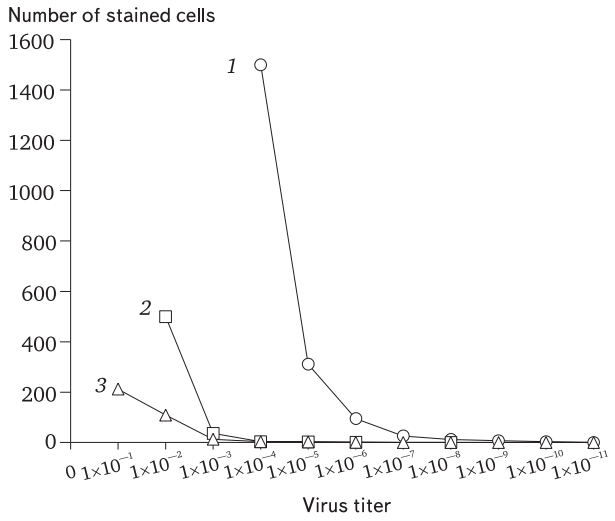

Fig. 2. Titration of infectious activity of AdSV40-βGal virus suspension in cell cultures of different types: MDCK (*1*), Vero (*2*), and HeLa (*3*).

method 3 weeks after cell injection into the myocardium.

Transplanted cells were detected at different periods after transplantation in cryostat sections by the standard histological method (staining for β-galactosidase).

RESULTS

In order to make sure that the created genetic construction can be effectively used for the detection of transplanted cells of different types, it was necessary first of all to determine the infective activity of the construction. To this end, AdSV40-βGal virus construction was added into various types of mammalian cell cultures: MDCK (dog kidney cells), Hela (human uterine epithelial cells), Vero (green monkey renal epithelial cells), and HepG2 (human primary hepatoma cells), and on day 5 after infection β-galactosidase activity was histochemically detected (Fig. 1). AdSV40-βGal construction infected different cell strains, but the expression of β-galactosidase was different in these cells. Vero cells proved to be the most sensitive system (Fig. 2). The next step in our study was the use of AdSV40-βGal construction for labeling primary cell cultures. The presence of β-galactosidase activity after cell culture infection was studied in the following cultures: primary human fetal brain neurons, fetal rat

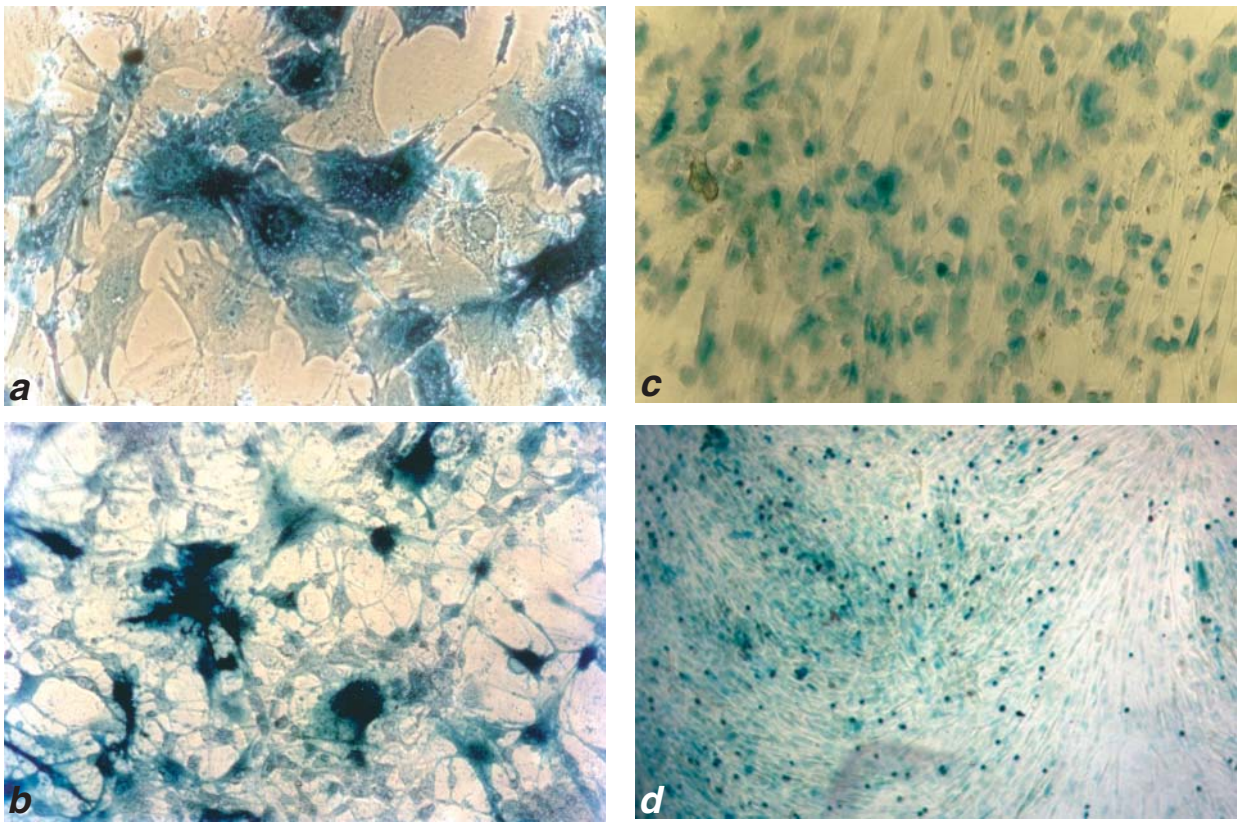

Fig. 3. Cultures of somatic cells infected with AdSV40-βGal and stained for β-galactosidase activity on day 5 after infection. *a*) primary culture of fetal rat cardiomyocytes, ×400; *b*) primary culture of human fetal brain cells, ×200; *c*) rat bone marrow cardiomyocyte-like mesenchymal stem cells (MSC) after 3-week culturing, ×200; *d*) human bone marrow cardiomyocyte-like MSC after 6-week culturing, ×50.

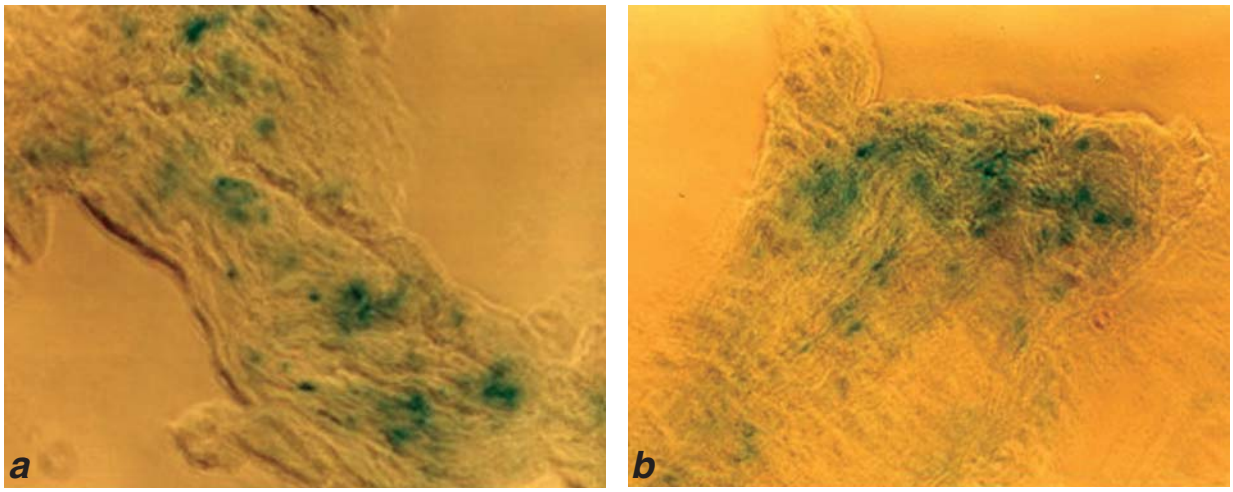

Fig. 4. Cryostat sections of myocardial left ventricle. Staining for β-galactosidase activity 21 days after transplantation into infarcted myocardial cicatrix. *a*) AdSV40-βGal-labeled rat bone marrow cardiomyocyte-like MSC, ×200; *b*) AdSV40-βGal-labeled fetal rat cardiomyocytes, ×200.

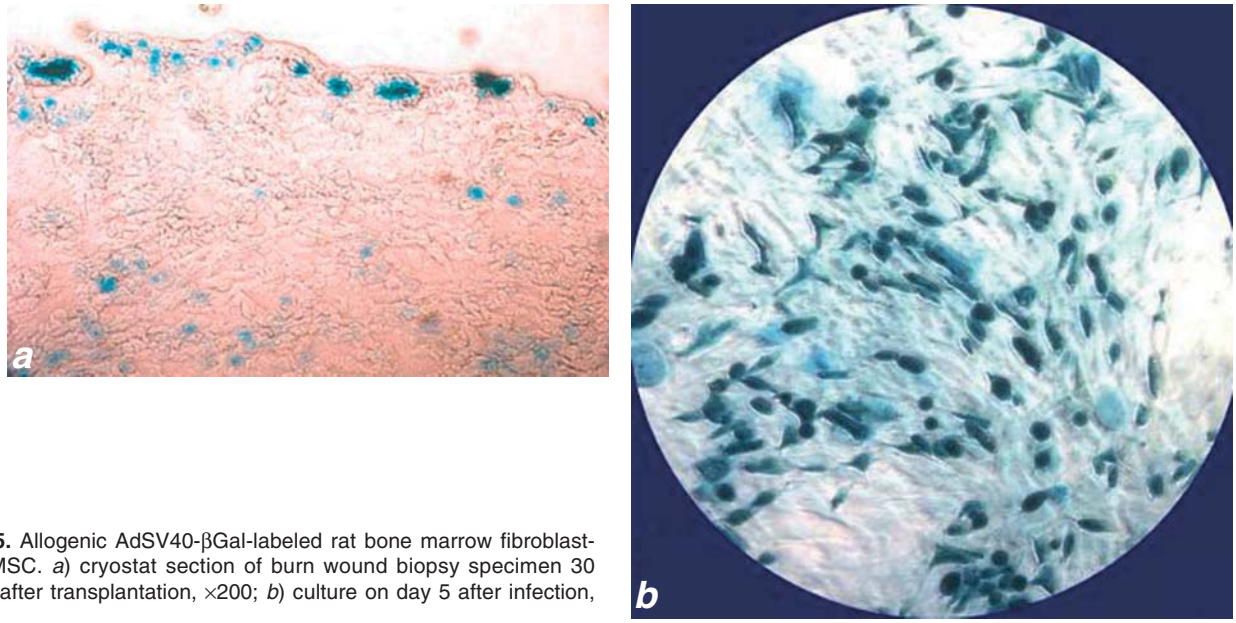

Fig. 5. Allogenic AdSV40-βGal-labeled rat bone marrow fibroblast-like MSC. *a*) cryostat section of burn wound biopsy specimen 30 days after transplantation, ×200; *b*) culture on day 5 after infection, ×400.

heart cardiomyocytes, and adult human and rat bone marrow MSC predifferentiated into cardiomyocyte-like cells (Fig. 3) [1]. These experiments showed that 15-20 AdSV40-βGal infectious particles per cell were needed for 80-90% cell infection in the absence of cytotoxic effect.

The authenticity of detection of exogenous *E. coli* β-galactosidase (Fig. 3) was confirmed by the absence of immunohistochemical staining of endogenous β-galactosidase, detected using monoclonal murine antibodies to *E. coli* β-galactosidase in cell culture and tissue sections.

The experiments showed that this viral construction can be used for monitoring of the transplanted cells. Experiments on rat model of myocardial infarction showed that 3-5 weeks after transplantation of fetal cardiomyocytes and bone marrow cardiomyocyte-like MSC, cells with β-galactosidase activity were detected in the perinecrotic zone (Fig. 4). The detection of β-galactosidase activity in the myocardium after transplantation of labeled cells coincided with improvement of the contractile function of damaged myocardium, which was attributed to the presence of transplanted cells in the myocardium.

Experiments with transplantation of labeled cells (fetal fibroblasts and fibroblast-like MSC) on the surface of burn wounds also showed the presence of cells containing bacterial β-galactosidase in the burn wound within 30 days after transplantation. The presence of labeled cells coincided with acceleration of burn wound healing, which led us to a conclusion on stimulation of the reparative process by transplanted cells (Fig. 5).

Hence, the described AdSV40-βGal construction is convenient for marking mammalian transplanted cells, including stem cells, but not hemopoietic cells. This construction can in a dose-dependent manner infect adhesive cells of various types; it is not toxic for these cells and for animals injected *in vivo*. It was also shown in animal experiments that the genetic construction used in this study detects the transplanted cells in the foci of injury during the period of up to 5 weeks after transplantation and the positive impact of cell therapy on tissue regeneration processes can be attributed to the presence of transplanted cells in the zone of injury.

We believe that this genetic construction can serve the base for creating a set of vectors for gene-therapeutic correction of many diseases.

REFERENCES

1. V. I. Shumakov, N. A. Onishchenko, M. E. Krasheninnikov, *et al.*, *Byull. Eksp. Biol. Med.*, **135**, No. 4, 461-465 (2003).
2. C. Cepko, E. Ryder, D. M. Fekete, and S. Bruhn, *Cells: A Laboratory Manual*, Eds. D. L. Spector *et al.*, Section II: *Localization of Organelles. Proteins and Gene Expression*, New York (1998), Vol. 3.
3. H. Goto, F. D. Shuler, C. Lamsam, *et al.*, *J. Bone Joint Surg. Am.*, **81**, 918-925 (1999).
4. A. J. Holtmaat, W. T. Hermens, A. B. Oestreicher, *et al.*, *Brain Res. Mol. Brain Res.*, **2**, 248 (1996).
5. E. H. Javason, D. C. Colter, E. J. Schwartz, and D. J. Prockop, *Stem Cells*, **19**, 219-225 (2001).
6. L. A. Kirshenbaum, W. R. MacLennan, W. Mazur, *et al.*, *K. Clin. Invest.*, **92**, No. 1, 381-387 (1993).
7. G. R. Mac Gregor, *Methods in Molecular Biology*, Ed. E. J. Murphy, New York (1991), Vol. 7, P, 217.
8. T. Muramatsu, Y. Mizutani, Y. Ohmori, and J.-I. Okumura, *Biochem. Biophys. Res. Commun.*, **230**, 376-380 (1997).
9. K. Nakano, M. Migita, H. Mochizuki, and T. Shimada, *Transplantation*, **71**, No. 12, 1735-1740 (2001).
10. E. M. Rust, M. V. Westfall, and J. M. Metzger, *Mol. Cell Biochem.*, **181**, Nos. 1-2, 143-155 (1998).
11. E. M. Rust, M. V. Westfall, L. C. Samuelson, and J. M. Metzger, *In Vitro Cell Dev. Biol. Anim.*, **33**, No. 4, 270-276 (1997).
12. G. Salvatori, L. Lattanzi, M. Coletta, *et al.*, *J. Cell Sci.*, **108**, 2733-2739 (1995).
13. A. Shaked, M. E. Csete, K. E. Drazan, *et al.*, *Transplantation*, **57**, No. 10, 1508-1511 (1994).
14. J. Shen, N. Taylor, L. Duncan, *et al.*, *Br. J. Ophthalmol.*, **85**, 861-867 (2001).
15. M. H. Soonpaa, G. Y. Koh, G. M. Klug, and L. J. Field, *Science*, **263**, 98-101 (1994).
16. R. Stripecke, M. Carmen Villacres, D. C. Skelton, *et al.*, *Gene Ther.*, **6**, 1305-1312 (1999).
17. S. Teramoto, H. Ito, and Y. Ouchi, *Thromb. Res.*, **93**, No. 1, 35-42 (1999).
18. D. C. Young, S. D. Kingsley, K. A. Ryan, and F. J. Dutko, *Anal. Biochem.*, **215**, No. 1, 24-30 (1993).