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ADENOVIRAL VECTORS: CONVENIENT TOOLS FOR GENE DELIVERY TO PRIMARY MAMMALIAN CELLS

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Tissue cell culture is widely used as convenient technique in modern biology and drug discovery. Multiple cell lines derived from normal and neoplastic tissues are used world-wide. However all the established cell lines, including pseudo-normal ones, are at least minimally transformed and frequently cannot properly re-capitulate the *in vivo* situation. Many of these limitations can be circumvented by using primary cells. Unfortunately, these cells are frequently difficult to transfect. The E1/E3-deficient recombinant adenoviruses shown to be highly efficient gene delivery tools that allow successful work with multiple types of difficult to transfect cells in different *in vivo* applications. Generation of the recombinant adenoviral constructs includes sub-cloning of the insert into a shuttle vector with convenient polycloning site with subsequent homologous recombination of the shuttle vector with an adenoviral backbone. We described a generation of more than 80 new rAdvs including expression, luciferase reporter and small hairpin interfering RNA vectors by using the AdEasy system. The generated recombinant adenoviruses can be used in multiple biological applications and in combination with proper maintenance protocol allow for 100% efficiency of transduction of difficult to transfect cells including primary mouse keratinocytes, human bone marrow-derived primary mesenchymal stem cells and other types of primary mammalian cells.

Key words: adenoviral vector; transduction, primary mammalian cells, mesenchymal stem cells.

Treatment of multiple diseases including inheritable, infectious, and geriatric disorders as well as a cancer creates a desperate need in generation of efficient vaccines and safe gene therapy tools and approaches. Modern biotechnology products used as the drugs improve a quality of life of patients suffering from rheumatoid arthritis, cancer and genetic diseases. In an addition the efficiency of gene delivery to different cell models is a limiting factor in the studies performed with difficult-to-transfect cells. The E1/E3-deficient recombinant adenoviruses (rAdv) were found to be easy to use and highly efficient tools for gene delivery to multiple types of difficult-to-transfect cells, including primary cells of epithelial and mesenchymal origin. Interestingly, injection of rAdv preparations into a tail vein of experimental mice leads to accumulation of up to 98% of vector in Kupffer cells in a liver [1, 2], thus providing a convenient selective tool to study biology of this organ. A great advantage of adenoviral vectors is similar to lentiviral vectors [3–5] capability to transduce cells independently of cell division rate. Adenoviral approach also allows reaching of the 100% transduction efficacy without influencing a viability of the cells.

Adenoviral transduction is transient and virus works ectopically, without integration into genome. First signs of transgene expression are evident as early as 8 hours after transduction, while reaching a maximum of expression round 36–48 hours after infection, afterwards staying stably high for next two weeks and slowly decreasing later due to dilution of a vector with every cell division. In some models of slowly or non-proliferating cells a significantly high expression of transgene can be observed for several months or even years in specific cases when immune system is impaired and cannot clear the infection [2].

Simplified Ad5-based AdEasy system that uses the homologous recombination in the bacterial cells [1] allows to perform in less than one month all the procedures starting from cloning to generation of high-titer rAdvs stocks. AdEasy system uses different shuttle vectors thus allowing a high versatility of functional cassettes integrated into rAdv cosmids. A deletion of E1, E2 and E3 regions of adenovirus (Adv) makes recombinant vectors replication deficient, thus reducing their biohazard level. Therefore amplification of the viral particles should be performed in special packaging cell lines (like human embryonic kidney HEK293, human retinoblastoma 911 [6] or Per.C6 cells [7]) that provide *in trans* missing protein products encoded by E1 and E3 regions. Size of viral capsid allows inserts ranging from 5 to 7.5 kb to be efficiently packed depending on the presence of promoter region and its type (particularly its size). For bigger sized inserts (up to 10.2 kb) the alternate system with missing E4 region was developed [1]. In such case missing E4 should be additionally provided *in trans* by packaging cell, for example, 911E4 cells that express E4 under control of *tet*-repressible promoter.

Currently many laboratories, including us, successfully use adenoviral approach with established cell lines. Due to low toxicity level, an infection with adenoviral vectors does not block a capability of transduced cells to differentiate into different lineages, thus being an additional advantage of adenoviral approach. Such feature while being combined with the high efficacy of gene delivery to both established cell lines and primary cells of different tissue origin makes adenoviral vectors the promising instruments in functional *in vitro* and *in vivo* studies and gene therapy tools in vaccination and treatment of inheritable diseases and cancer. To date available in literature data describing the use of adenoviral vectors aiming to transduce human mesenchymal stem cells (hMSC) is rather controversial and some laboratories claim that these cells are not susceptible for Ad5-based vectors.

The main goal of current study is to generate an efficient set of tools that allows a high versatility of modern molecular biology approaches in studies performed with the primary cells, including mesenchymal stem cells, and to experimentally validate the practical use of the created tool set.

Materials and Methods

Cell culture and ligands. Human bone marrow (BM)-derived mesenchymal precursor cells and primary fibroblast-like synoviocytes (FLS) were obtained under patient consent agreement correspondingly from BM aspirates or arthroscopy biopsies according to IRB protocol. Cells were separated using classic Ficoll gradient and cultured in growth medium consisting of low glucose Dulbecco's modified Eagle's medium (DMEM, Sigma) supplemented with 10 ng/ml of basic fibroblast growth factor (Peprotech) and 10% fetal bovine serum (FBS, Sigma, Germany) in 5% CO_2-containing atmosphere at 37 °C. For adenoviral infections cells were transferred to DME supplemented with 4% FCS and 16 hours later transferred to fresh DMEM/10% FCS supplemented with appropriate ligands or vehicle buffers. Primary mouse keratinocytes were isolated from newborn wild type C57BL/6 mice and cultured in serum-free keratinocyte medium (Invitrogen) according to previously published protocol [8]. Recombinant BMP2 and TGFβ1 were from Peprotech.

Generation of recombinant adenoviruses. rAdvs were generated using AdEasy system according to provided protocol [1]. Briefly, the cDNA of gene-of-interest was subcloned into the pShuttle-CMV vector in case of generation of a gene expression vector. For generation of shRNA (small hairpin interfering RNA) vectors a cassette including H1 promoter and shRNA expressing insert was recloned from a pSUPER construct into pShuttle plasmid. Generation of luciferase reporter vectors was carried out by recloning the cassette including a pathway-specific promoter, luciferase CDS and following polyA signal from pGL3 vector into pShuttle. The constructs obtained were recombined with the Easy-1 adenoviral backbone in BJ1583 cells. The linearized cosmids were transfected into 293 cells using FuGENE 6 (Roche) or Gene Juice (Novagen) transfection reagents and amplified as Advs. Viruses were titrated in transcomplemental 293A cells and purified with cesium chloride gradient ultracentrifugation as described previously [1].

Fluorescence-activated cell sorting (FACS) analysis

hMSC cells were harvested by trypsinization and incubated on ice with fluorochrome-conjugated antibody for 20 minutes while keeping samples protected from a light. Afterwards cells were rinsed twice with ice-cold PBS and fixed with ice-cold, freshly prepared 2% paraformaldehyde (Sigma-Aldrich, St Louis, MO, USA). FACS analysis was carried out using a Becton-Dickinson Immunocytometry System and a FACS Calibur cytometer (Becton Dickinson, San Jose, CA, USA) with minimal

counts of 10,000 events. The following human antibodies labeled with fluorescein isothiocyanate (FITC) or phycoerythrin (PE) were used: major histocompatibility complex (MHC) class I (HLA-ABC)-FITC, CD13-PE, CD29-PE, CD31-FITC, CD34-FITC, CD44-PE, CD90-FITC (BD Pharmingen Inc., San Diego, CA, USA), CD105-PE (eBioscience, Inc., San Diego, CA) and MHC class II (HLA-DR)–PE (CLB, Amsterdam, The Netherlands).

Transcriptional reporter assays. Primary mouse keratinocytes were adhered to a bottom of wells in 12-well plates (Corning, France). Next day cells were infected with the indicated rAdvs at MOI = 50. β-galactosidase-expressing rAdv was co-infected at multiplicity of infection (MOI) = 5 to be used as an internal control for normalizing infection efficiency. Luciferase and β-galactosidase activity were quantified using Luciferase assay (Promega) with Autolumat Berthold luminometer and Emax 96-well plate reader (Molecular Devices) correspondingly as described previously [9]. All the experiments were performed in triplicates and repeated minimally tree times. Data from representative experiment are expressed as a general mean with standard deviations.

Alkaline phosphatase assay. Histochemical examination of alkaline phosphatase activity produced by hMSC was performed using naphtol AS-MX phosphate (Sigma, St. Louis, MO, USA) and fast blue RR salt (Sigma, St. Louis, MO, USA) as previously described [10, 11]. Cells were seeded in 12-well plates at 60% confluency (2×10^4/cm^2). Next day cells were infected with adenoviral vectors at MOI = 200. Seven hours later cells were washed once with phosphate buffered saline (PBS), pH 7.4 and cultured in DMEM/10% FCS supplemented with 0.2 mM of ascorbic acid (Sigma, St. Louis, MO, USA). Alkaline phosphatase activity was measured after 4 days of culture.

Results and Discussion

To address research and potentially future gene therapy needs in the efficient tools for gene delivery to stem cells, including hMSC, we generated more than 80 different rAdv constructs using AdEasy system including those that are mentioned in Table. These vectors include different expression constructs, particular signalling pathway-specific luciferase reporter constructs and small hairpin interfering RNA (shRNA) vectors for the knockdown of gene expression. Several of mentioned above constructs have been already published with in vitro cell lines [12, 13].

The important step in the amplification of rAdv is an efficient transfection of linearized rAdv cosmid, sized over 35 kb, into a packaging cell line (HEK 293 or 911 [6] cells). It is well known that both HEK 293 or 911 cells are weakly adhered cells. Therefore many of efficiently transfected cells are lost during the multiple changes of culture medium that are required according to the Lipofectamin-based transfection protocol. The use of low-toxicity transfection reagents like FuGene6 (Roche) and GeneJuice (Novagen) allows to avoid excessive changes of culture medium and to accelerate the initial step of rAdv amplification. According to literature in most of adenoviral stocks only one out of 20 particles is infectious and completely active. In our hand we usually get such ratio round 1/8 and preparative cultures prepared on 10×140-mm Petri dishes allows us to get 1×10^{11}– 2.5×10^{12} of infectious viral particles when monitored using a convential plague forming assay [1, 14]. Usually yield depends on multiple factors, including a density of cells at the moment of infection, age of the culture, and a number of re-amplification steps viral stock went through. Due to the presence of E1 regions stably integrated into genome of packaging cell line growing numbers of re-amplification steps lead to accumulation of unwanted recombination events resulting in a loss of transgene expression. In our experience we did not observe a significant loss of transgene expression in up to 8 re-amplification steps when we used single insert vectors. At the same time already a 6th re-amplification step sometimes yielded a lower transgene expression with inserts placed in pAd-Track system due to

Recombinant adenoviral constructs generated at our lab

Expression rAdv	shRNA-expressing rAdv that target:	Luciferase reporter vectors
p65/RelA, mTIEG, hTIEG, mCXCR1, hSpp2, mSpp2, hHSP72, RUNX2, IKKα-AA, IKKβ-AA BMP2, BMP7, mWnt3a, hPADI2, hPADI4	mMyc (LZ domain), mMyc (TAD domain), h-p65/RelA, hSmad2, hSmad3, hSmad5, mTIEG, hTIEG, hAUF1, hBRF	BRE-Luc (BMP-Smad-specific) $(CAGA)_{12}$-Luc (TGFβ-Smad-specific) 4xNF-κB-Luc (NF-κB-specific) Bat-Luc (β-catenin/TCF4-specific) 4xHSE-Luc (Heat shock element) RUNX2-specific (6xOSE2-Luc, osteoblast-specific)

homologous recombination between two analogous CMV promoters placed in head-to-tail orientation with one promoter driving an enhanced Green fluorescent protein (eGFP) expression and an other one controlling transgene expression [1].

The primary mouse keratinocytes, the BM-derived primary hMSC and hFLS are all well known as difficult-to-transfect cells. Actually main problem with hMSC and hFLS is not a bad delivery of plasmid DNA to the cells, but a fact that all efficiently transfected cells are going to die shortly (within 2–3 days) and therefore cannot be used for any sort of informative studies. High efficiency of rAdv gene delivery in hMSC allows us to get up 100% of transduction at low multiplicity of infection (MOI) = 100 (Fig. 1, *A*). Interestingly, hMSC can tolerate high titers of rAdv (up to MOI = 10,000) without significant cell toxicity (less that 3% of dying cells when monitored with classical trypan blue staining; data not shown). Convential transfection procedure does not allow getting of 100% of transduced cells in transient assays and stable transfection with subsequent antibiotic selection is usually needed for getting of more homogeneous population of transduced cells. Taking into account that the efficiency of such integration usually ranges round 0.001–0.02%, this approach cannot be used with primary cells that are usually available in limited numbers. Such limitation can be also circumvented with lentiviral transduction [3–5] as an alternative technique, however a regular lentiviral preparation yields much lower titres of the virus, thus being a technical limitation with this approach.

Induction of osteoblast differentiation in hMSC. Adenoviral delivery of constitutively active (ca) bone morphogenetic protein (BMP) type I receptor called activin-like kinase 2 (ALK2) into hMSC induces and potentiates their osteoblast differentiation observed with histochemical staining for alkaline phosphatase (EC 3.1.3.1) activity that is a marker for early stages of osteoblast differentiation (Fig. 1, *B*) [10, 11].

Interestingly, the infectability and therefore usefulness of adenoviral approach depends on the protocol of hMSC maintenance. We have a very good experience with our protocol for isolation and maintenance of hMSC from human BM. According to our protocol we use 10 ng/ml of bFGF as a supplement to culture medium. Other laboratories use a lower concentration of bFGF (1 ng/ml) in a growth (propagation) medium. Cells isolated and maintained with both mentioned above protocols preserve a capability to give rise to different types of mesenchymal cells, including myoblasts, adipocytes, chondroblasts and osteoblasts (Fig.1 and data not shown). We performed a FACS analysis of obtained hMSC population after five one-to-three splittings.

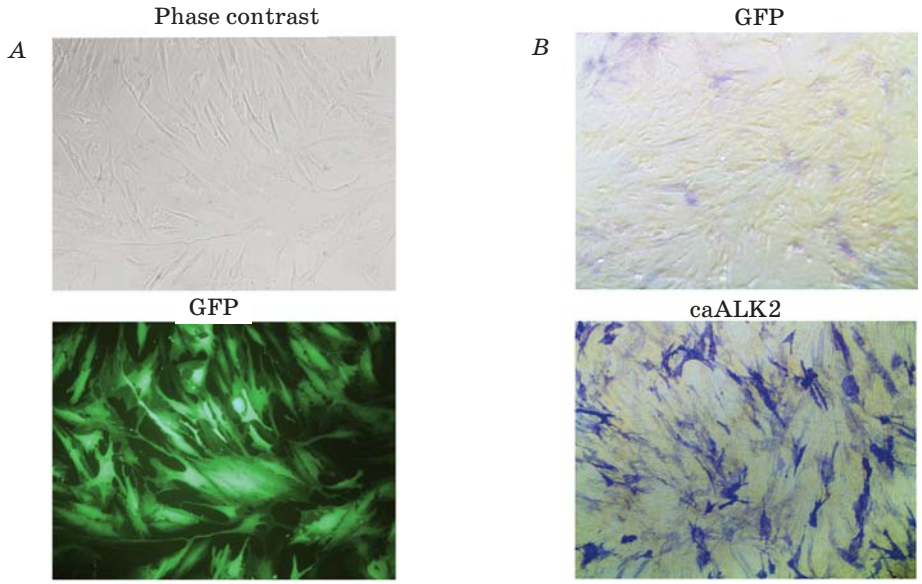

Fig. 1. **Adenoviral transduction is an efficient approach to get 100% of transduced cells and to induce early osteoblast differentiation in the primary human mesenchymal stem cells (hMSC):**

A — hMSC at density of $1.5\times10^4/cm^2$ were infected with recombinant green fluorescent protein (GFP) adenovirus at a multiplicity of infection (MOI)=100. Upper panel: phase contrast. Lower panel: GFP fluorescence.

B — Alkaline phosphatase activity (blue) in hMCS upon adenoviral transduction with control GFP or constitutively active (ca) bone morphogenetic protein type I receptor ALK2 adenovirus (MOI=500). Representative fields (100x) are shown (*A* and *B*)

As a result we confirmed expression of CD13 (Alanine aminopeptidase, zink metalloprotease, EC 3.4.11.2) [15, 16], CD44 (hyaluronic acid receptor, a cell-surface glycoprotein that functions as an adhesion molecule) [17–19], CD73 (ecto-5'-nucleotidase, that converts AMP to purine nucleotide, EC 3.1.3.5) [20, 21], CD90 (Thymocyte antigen 1, Thy-1, a cell surface glycoprotein) [18, 22], and CD105 (also called endoglin, a TGFβ and BMP type III receptor) [23–25], HLA-ABC (human leukocyte antigen class A, B or C; corresponding to MHC class I) that are well accepted markers of mesenchymal stem cells. As expected, the cells were negative for hematopoietic marker CD34 [26, 27], the costimulatory adhesion molecule CD31 (platelet/endothelial cell adhesion molecule, PECAM-1) [28, 29], CD29 [30, 31] (Fig. 2) and HLA-DR (HLA corresponding to MHC class II; data not shown) [27, 32, 33].

Reporter assays in primary cells. The luciferase reporter assay is easy to perform and widely used Fig. 2sed functional assay to investigate cell signalling pathways and their crosstalks. Adenoviral approach allows us to successfully investigate the transforming growth factor β (TGFβ), bone morphogenetic proteins (BMP) (Fig. 3), nuclear factor κB (NF-κB), wnt (β-catenin/TCF4) and RUNX2 driven transcription in the mouse primary keratinocytes, human BM-derived MSC and human fibroblast-like synoviocytes (data not shown).

Epidemiology of adenoviruses. Advs are non-enveloped (i.e. their capsid does not contain lipid components and virus therefore is not sensitive to sterilization with a 70% ethanol) DNA viruses with diameter approximately 90 to 100 nm. First identification of Advs was in 1953 when Rowe et al. [34] isolated these pathogens from human adenoids.

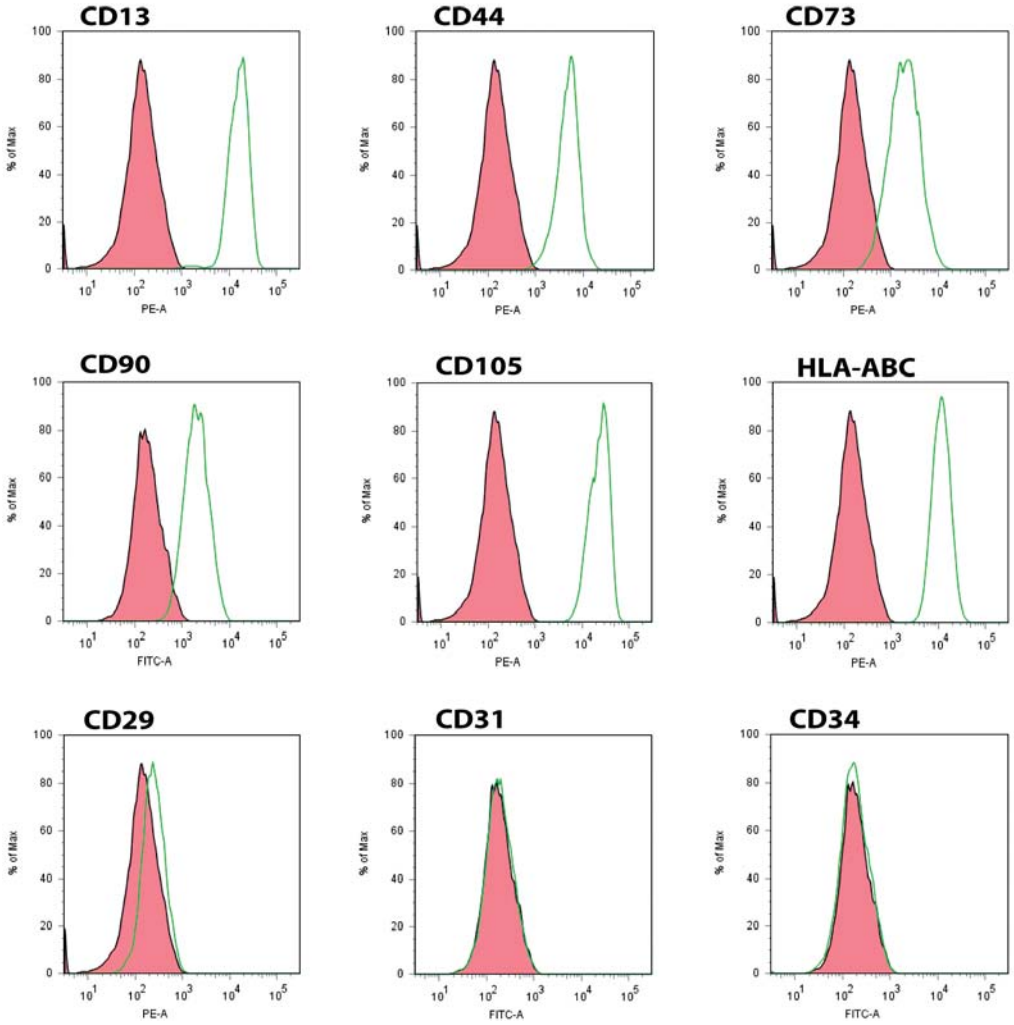

Fig. 2. **Immunophenotyping of hMSC cells:**
hMSC cells were harvested, immunostained with indicated fluorescein isothiocyanate (FITC) or phycoerythrin (PE)-conjugated antibodies, fixed and analysed by FACS (green line peaks). Same cells without an addition of antibody were used as the control (peaks filled with red)

Most of Adv serotypes cause respiratory diseases with mild symptoms [2, 34] while some others lead to acute respiratory pathologies (Ad3, 4, 7, 14, 21), keratoconjunctivitis (Ad8, 9, 10, 19), gastroenteritis (Ad40, 41) and even obesity (Ad36, 37) [2, 35–37].

Fifty five different serotypes of Advs are described to date [2]. According to current classification these serotypes were divided into seven species, from A to G. Ad2 and Ad5 that belong to species C are most widely distributed and therefore mostly well studied adenoviruses. According to evaluation performed by Holterman et al., [38] and d'Ambrossio et al. [39], 30–50% of Amerinans, 60% of Japanese and 45–75% of Europeans possess pre-existing immunity to Ad5. In sub-Saharan' Africa, India and Brasil practically all adult population has a history of Ad5 infections [2, 40–43].

Structure and delivery of adenoviruses to target cells. Advs are icosahedral (i.e. twenty facets-shaped) viruses with capsid formed by three major proteins: fiber, penton base and hexon. There are 36 monomers of fiber, 60 monomers of penton base and 720 monomers of hexon on each Adv virion [2]. Fiber monomers trimerize to form antenna-like structures that are placed at the each vertex of the capsid icosahedral. The tail domain of fiber trimers attaches directly to penton that forms a shaft adhering fibers to the icosahedral facets formed by the hexon trimers. Therefore fibers are most exposed and easily accessible structures on Adv virion that are available for interactions with host proteins, thus most efficiently mediating virus attachment and infection on the host cell [44, 45]. Several less efficient and slower formed interactions are formed by penton proteins [46] and very little evidence is shown for hexon interactions with host proteins (for review see [2]).

Coxsackie-adenovirus receptor protein (CAR) due to its specific interaction with the fiber proteins of Adv capsid has been identified as the primary accessory receptor for the attachment of Ad2 and Ad5 to target cell and subsequent infection [47]). Therefore, the infectability of the target cell depends on the expression level of CAR, a transmembrane protein of 46 kDa that belongs to the immunoglobulin superfamily [48]. CAR is a cell adhesion molecule that is highly expressed on epithelial cells thus making these cells especially good target for adenoviral gene delivery. Mesenchymal cells express lower levels of CAR, therefore higher titers of rAdv are required for efficient transduction of cells.

Unfortunately, most of hematopoietic cells (B- and T-lymphocytes, monocyte/macrophages) do not express sufficient levels of CAR, thus being barely, or completely non-infectable with Ad5-based convential vectors. This limitation can be circumvented by studies performed with CAR-overexpressing cells (for example, derived from truncated hCAR recombinant mice [49]) or with novel recombinant vectors that have different accessory receptor determinants, for example pseudotyped with chimeric fiber domains [50]. The adenoviral vectors generated with this approach are also less immunogenic due to the absence of all entirely adenoviral proteins. Mentioned deletions also render these vectors

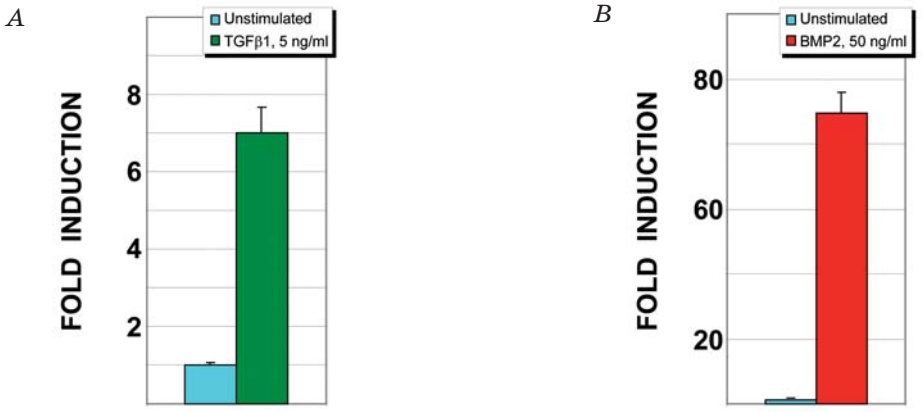

Fig. 3. **The TGF 1 and BMP2-driven activation of according $(CAGA)_{12}$-Luc (*A*) and (BRE)-Luc (*B*) luciferase reporter adenoviral constructs in the mouse primary keratinocytes:**
primary mouse keratinocytes were split into 12-well plates (a suspension from one newborn pup spread per two plates) and infected with indicated vectors at MOI=50. Folds induction with respectively TGFβ1 (5 ng/ml) or BMP2 (50 ng/ml) are shown. All the experiments were performed in triplicates and repeated minimally tree times. Data from representative experiment are expressed as a general mean with standard deviations

into high-capacity (hc) Adv vectors that allow big inserts or cassettes containing multiple transgenes [51]. Such approach allowed for gene delivery of 14 kb insert encoding dystrophin that was proposed for treatment of Duchenne muscular dystrophy (DMD) [50, 51]. The current technical disadvantage of this system is the absence of simplified, similar to AdEasy, system for generation of recombinant hcAdv.

An important modern application of Ads is their use as the vaccine vehicles to generate an immune protection against HIV and other infectious diseases. In studies performed with Rhesus monkey using homologous to HIV simian immunodeficiency virus (SIV) Liu et al. [52] overexpressed SIV gag protein to generate a T-cells-based protective immunity, thus reaching 1.4 to 2.4 log reduction in peak and chronic viremia levels. In this case a heterologous system by combining Ad26 and Ad5-based vectors was used. An advantage of Ad26 is its lower seroprevalence that is at a 26% level in Sub-Saharan Africa which is in a desperate need of HIV vaccine [2, 40].

Oncolytic adenoviruses. A modern approach uses genetically modified conditionally replication-competent (CRC) Adv vectors. In this method Adv genome still contains E1A region, but under control of heterologous, tumor-specific promoter [2, 53, 54]. As result recombinant Advs can replicate exclusively in target tumor cells, while being still recombination-deficient in normal cells, where tumor-specific promoter is silent. Amplification of CRC viruses in target tumor cells finally kills these cells leading to release of the virus and its further spreading on neighboring tumor cells, thus further amplifying the oncolytic effect. In real *in vivo* situation the efficacy of CRC-Adv therapy is still limited due to formation of necrotic areas and elimination of vector by host immunity. As a result combination with other therapeutic agents is usually required [53, 55].

Several clinical studies are currently performed aiming to generate tumor-targeting adenoviruses. Unfortunately, genetic elements encoding high-affinity molecules, like antibodies, due to their large size cannot be incorporated into viral genome for direct pseudotyping of viruses. In an addition, a reducing environment in the nucleus, where Ad is assembled, leads to improper folding of antibodies. A more promising approach is generation of adaptor molecules, like soluble CAR (sCAR) fused to targeting molecules like FGF-2, EGF [56], carcinoembryonic antigen (CEA) [57, 58] or folate [59, 60]. A main limitation of this approach is a fact that the mentioned above adaptor molecules are non-covalently bound to the viral capsid and *in vivo* such binding appears to be rather too weak [2].

Binz et al. [61, 62] generated a library of designed ankyrin repeat proteins (DARPins), cysteine-less alternatives of antibodies that consist of helical repeats-containing protein interaction interfaces that have been designed to bind Adv fibers. Similar DARPins library was further designed and screened for dual specificity to both fibers and target protein HER2 (Human Epidermal growth factor Receptor 2) — a molecular marker for a bad prognosis in breast, gastric and other cancers [53, 55, 63, 64]. Such fusion DARPin molecules with simultaneous fiber/HER2 binding can be produced in *E.coli* aiming to specifically target oncolytic Advs to highly malignant cancer cells. Currently additional studies are performed for *in vivo* validation and potential clinical use of dual DARPin approach [2, 64].

Mesenchymal stem cells as vehilcles for adenovirus delivery. It is well known, that circulating stem cells can be recruited from the blood into peripheral solid organs that require regeneration [65–68]. One more highly promising approach in tumor treatment was proposed based on the evidence suggesting that stem cells can serve as the useful delivery vehicles for brain tumors therapy [53, 55]. Two possible systems can be potentially used: neural stem cells and hMSC. First approach is based on the finding that intracranially injected neural stem cells have a tropism to brain tumors [69]. Later a therapeutic effect was showed using neural stem cells overexpressing interleukin-12 or tumor necrosis factor–related apoptosis inducing ligand (TRAIL) [70, 71]. Due to technical limitations with the availability of neural stem cells from patients, hMSC appeared as an easily accessible alternative to neural stem cells. hMSC can be easily obtained from the same patient thus making possible an autologous transplantation, that eliminates immunologic incompatibilities [72]. Using an intracranial model of gliomas, it was showed that hMSCs have a tropism for human gliomas after both intravascular or local delivery, and such tropism can be exploited therapeutically by using the hMSC as vehicles for adenoviral vectors [73]. This direction was further explored using hMSCs as vehicles for replication-competent oncolytic Advs [55]. In this study virus-loaded hMSCs effectively migrated *in vitro* and released Advs that infected U-87MG mouse glioma cells. Strikingly, hMSCs injected away from the tumor site *in vivo*,

efficiently migrated to the tumor and delivered the viral payload to its specific target [55].

Numerous adenoviral approaches that allow achieving 100% efficiency of transduction of resistant to transfection cells, including mesenchymal stem cells, were described and approved in multiple biological applications. Such a very high efficacy of the primary cells transduction allows usage of adenoviruses as gene delivery tools in research and therapeutic applications for treatment of genetically determined disorders, as vaccine vehicles or oncolytic adenoviral vectors generate a modern promising therapeutic approach to treat various diseases. The generated set of adenoviral gene delivery and research tools provides a perfect platform for modulation of function and studying mechanisms of signal transduction in various primary mammalian cells.

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АДЕНОВІРУСНІ ВЕКТОРИ: ЗРУЧНІ ІНСТРУМЕНТИ ДЛЯ ДОСТАВЛЕННЯ ГЕНІВ У ПЕРВИННІ КЛІТИНИ ССАВЦІВ

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Культуру тканин широко застосовують як зручний метод сучасної молекулярної біології та для створення лікарських препаратів. В усьому світі використовують численні лінії клітин, отриманих з нормальних і пухлинних тканин. Однак усі існуючі клітинні лінії, у тому числі псевдонормальні, є принаймні мінімально трансформованими, а дослідження, що проводяться з їх використанням, часто не можуть належним чином відтворювати ситуацію, яка існує в природних умовах. Багатьох із цих обмежень можна уникнути за допомогою первинних клітин. На жаль, ці клітини погано піддаються трансфекції. У різних лабораторіях E1/E3-дефіцитні рекомбінантні аденовіруси було охарактеризовано як високоефективні інструменти доставлення генів, які в умовах *in vivo* дають змогу успішно працювати з різними типами клітин, що важко піддаються трансфекції. Генерування рекомбінантних аденовірусних конструкцій передбачає субклонування вставки до зручної рестрикційної ділянки човникового вектора з наступною гомологічною рекомбінацією отриманого вектора з основною частиною аденовірусного геному. У роботі описано створення за допомогою AdEasy системи понад 80 нових rAdvs, зокрема експресійних, люциферазних репортерних систем і векторів, що містять малі інтерферуючі шпилькові РНК. Створені рекомбінантні аденовіруси можуть бути використані в багатьох біологічних системах і в поєднанні з належною процедурою підтримання культури дають можливість досягти 100%-ї ефективності трансдукції клітин, що погано піддаються трансфекції, зокрема первинних кератиноцитів миші, первинних мезенхімних стовбурових клітин кісткового мозку людини та інших типів первинних клітин ссавців.

Ключові слова: аденовірусні вектори, трансдукція, первинні клітини ссавців, мезенхімні стовбурові клітини.

АДЕНОВИРУСНЫЕ ВЕКТОРЫ: УДОБНЫЕ ИНСТРУМЕНТЫ ДЛЯ ДОСТАВКИ ГЕНОВ В ПЕРВИЧНЫЕ КЛЕТКИ МЛЕКОПИТАЮЩИХ

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Культуру тканей широко применяют в качестве удобного метода современной молекулярной биологии и для создания лекарственных препаратов. Во всем мире используется множество линий клеток, полученных из нормальных и опухолевых тканей. Однако все существующие клеточные линии, в том числе псевдонормальные, являются по крайней мере минимально трансформированными, а исследования с их использованием часто не могут должным образом воспроизводить ситуацию, существующую в естественных условиях. Многих из этих ограничений можно избежать с помощью первичных клеток. К сожалению, эти клетки плохо поддаются трансфекции. В разных лабораториях E1/E3-дефицитные рекомбинантные аденовирусы были охарактеризированы как высокоэффективные инструменты доставки генов, которые в условиях *in vivo* позволяют успешно работать с различными типами клеток, трудно поддающихся трансфекции. Создание рекомбинантных аденовирусных конструкций включает в себя субклонирование вставки в удобный рестрикционный участок челночного вектора с последующей гомологичной рекомбинацией полученного вектора с остальным аденовирусным геномом. В работе описано создание с помощью AdEasy системы свыше 80 новых rAdvs, включая экспресионные векторы, люциферазные репортерные системы и векторы, содержащие малые интерферирующие шпилечные РНК. Созданные рекомбинантные аденовирусы могут быть использованы в различных биологических системах и в сочетании с соответствующей процедурой поддержания культур позволяют достичь 100%-й эффективности трансдукции клеток, плохо поддающихся трансфекции, в том числе первичных кератиноцитов мыши, первичных мезенхимных стволовых клеток костного мозга человека и других типов первичных клеток млекопитающих.

Ключевые слова: аденовирусные векторы, трансдукция, первичные клетки млекопитающих, мезенхимные стволовые клетки.