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Cultural Properties of Viruses and Design of Safe Recombinant Vaccines and Virus Gene Constructs on their Basis

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Review Article

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Abstract

The results from various studies have shown a lot of particular differences in the mean length of telomere regions of chromosomes from stem cells at different stages of development and differentiation. The differences between stem/progenitor cell types in DNA-repair and telomere maintenance pathways have been found as co-evolved with the cell proliferation, differentiation, transformation, aging and life span. Studies on these cells are often focused on their ability for self-renewal. On the other hand, however, like damages in these processes have been characterized cancer and leukemia. Hence, the detailed study of these processes is necessary for understanding of the mechanisms, leading to repair and regeneration of mutated, damaged and/or destroyed cells, tissues and organs. As a new perspective in this aspect has been established the use of cell vectors, obtained by transduction of genes in stem/progenitor cells, by virus gene constructs. Avian pox viruses (APVs) can replicate in different cell-culture systems: primary embryo cells, diploid cell clones and permanent cell lines. The needs for production of more safety vaccines have challenged the research to find suitable cell-culture systems for their cultivation. In the last years abilities for replication of these viruses in non-avian cells and their possible application for vaccination of non-avian species were revealed. Because that, different cell-culture systems have been used for production of recombinant APVs (rAPVs) as a base for construction of recombinant vaccines against different diseases in birds and mammals, including people. In such way, the avian cell-culture systems could be used for production of rAPV vaccines against diseases in birds, and the mammalian cell-culture systems – for production of such vaccines against diseases in mammals. In addition, the permanent cell lines possess a lot of advantages in comparison with the primary embrional cells and could be also used for isolation and detection of APVs, as well as for diagnostic aims. Techniques, developed for construction of recombinants of vaccinia virus, could be adapted in the construction of recombinant avian pox viruses (rAPVs), especially of recombinant fowlpox viruses (rFWPVs) and

recombinant canarypox viruses (rCNPVs). These abilities make possible event the possibility of treatment of some types of tumors in people.

Keywords: Cell Cultures; Cell Lines; Stem/Progenitor Cells; Avian Pox Viruses; Recombinant Avian Pox Viruses; Vaccines; Virus Gene Constructs

Abbreviations: APVs: Avian Pox Viruses; RAPVs: Recombinant Avian Pox Viruses; RCNPVs: Recombinant Canarypox Viruses; T-Ctl: T-Cytotoxic Lymphocytes; CEFs: Chicken Embryonic Fibroblasts; APVs: Avian Pox Viruses; FwPV: Fowl Pox Virus CnPV: Canarypox Virus; PgPV: Pigeon Pox Virus; PCR: Polymerase Chain Reaction; T-Ctl: T-Cytotoxic Lymphocytes; HSV: Herpes Simplex Virus; HTERT: Human Telomerase Reverse Transcriptase; HDMECs: Human Dermal Microvascular Embryonic Cells; SCID: Severe Combined Immunodeficiency; CCs: Cell Cultures.

Introduction

Studies on the biology of the stem/progenitor cells are often focused on their self-renewal and differentiation [1]. It is important to note that the efficiency of DNA-repair varies greatly among different stem cell types [2]. Stem cells in young and middle-aged mice have been found to be rarely in the $S/G_2/M$ phases of the cell cycle, but in old mice - frequently in the cycle [2,3]. One of the possible hypotheses has been that the unexpected proliferation in old mice might be related to the increased incidence of leukemia in old mice. Hence, these cells change with age. The high self-renewal potential of the stem/progenitor cells in *in vitro*-conditions makes them strong candidates for delivering of genes, as well as for restoring organ systems function have been found to be included in these processes. This understanding could be applied toward the ultimate goal of using stem/progenitor cells not just for various forms of therapy, but rather as a tool to discover the mechanisms and means to bring, reconstituting them from old and young individuals has exhibited indistinguishable progenitor activities *in vivo* and *in vitro* [4,5]. In using of experimental mice, it has been determined that, after mobilization with receptor antagonist, exited from bone marrow stem/progenitor cells, have transited blood and engraft in open niches in partner marrow. The niche has been proposed as an environment that insulates stem cells from various stimuli and maintains them in a quiescent state [6]. The properties of "malignant stem cells", have outlined initial therapeutic strategies against them.

The differences between stem/progenitor cell types in DNA-repair and telomere maintenance pathways have been found as co-evolved with cell proliferation, transformation, aging and life span [3,4]. This idea has given rise to the notion that many aspects of normal aging could primarily reflect limitations in DNA-repair and telomere-maintenance pathways in the stem/progenitor cells [7]. In humans, the levels of enzyme telomerase in the stem cells have been established under extremely tight control, as it has been illustrated by the bone marrow failure in patients with telomerase deficiencies. In the biology of human stem/progenitor cells, telomerase has been proposed to play an important role in the repair of guanine-rich DNA-regions [4]. These cells have been defined as pluripotent, with self-renewal capacity [3,4,7]. Recent studies have shown striking differences in the mean length of the telomere repeat sequences at the chromosomes' ends from human stem/progenitor cells at different stages of development [3,4,7,8]. The most likely expansion for these observations has been that stem/progenitor cells, which, like all other somatic cells, lose telomere DNA upon each cell division. Clinical use of cultured human mesenchimal stem cells has begun for cancer patients and the recipients have received autologous or allogeneic stem cells [9 -11].

The necessity of production of more secure and safer vaccines against the poxvirus diseases imposed studies for searching of suitable cell-culture systems for replication of these viruses. For the first time such virological method has been suggested in 1949. A process of propagation of fowl pox virus (FWPV) in cell cultures (CCs) with epithelial origin, seeded as roller cultures has been established, and the authors have observed osmiophilic cytoplasmic inclusions [12-14]. This process has been confirmed by the death of the cells for some days and also by the high virus titer in the supernatant. The same authors have done a message about the propagation of FWPV in chicken embryonic fibroblasts (CEFs), seeded as roller cultures [15]. The roller cultures are suitable for cultivation and propagation of avian pox viruses (APVs), because the constantly change of the liquid and the air phase ensures more favorable conditions for virus replication. The changes, which have been observed in the

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roller cultures, have been the same, which were characteristic for CCs, infected in monolayer (specific cytoplasmic inclusions, vacuolization of the cytoplasm, rounding of the cells and their detachment from the substrate). One of the most important factors is the rolling speed. For example, the amount of produced by vaccinia virus pathological changes in cultures, rolled at 96 rpm, has been significantly enhanced in comparison with cultures, rolled at 2 rpm or held stationary [12-15].

Vaccines, based on virus genome constructs, have been found to suggest new possibilities for effective control of different diseases in birds and mammals, including humans [16-23]. Both RNA- and DNA-viruses could be used as virus vectors. Virus vector vaccines, received by insertion of genes, which code define sequences of different viruses and epitopes, have possessed a lot of advantages in comparison with the attenuated, died and subunit vaccines. The fact that the recipients could be immunized against specific antigens has been characterized as one of the most important features of the live virus vectors. Possible modifications have been proven to be an increasing of the expression level of the foreign antigen by a change in the promoter or in the insertion site, in the target repeats of the virus vector, in the expressing antigen and/or in the co-expressing immunomodulator proteins [19 -22]. Because of the large size of the genome, the pox viruses have been found to be able to express more than one foreign gene for creation of polyvalent vaccines, as well as some non-essential genes and some regulating sequences in the genome of the fowl pox virus (*FWPV*) [20]. As a necessary has been established the insertion of foreign genes to be done on such way that the replication of the parent virus not be concerned, but on the other hand, the last has been characterized as necessary for an optimal expression of the inserted genes [24-28].

Cultivation of Avian Pox Viruses in Cell Cultures

A successful cultivation of *FWPV*, pigeon pox virus (*PGPV*) and canarypox virus (*CNPV*) has been done in mammalian CCs was done after consistent adaptation passages in chicken and duck embryo cells [16-54]. CCs have also been used for differentiation of *APVs* (in antigenic composition, as well as in genetic respect), for diagnostic purposes and for production of recombinant *APVs* (*rAPVs*) [18,20-40,44,46-54]. An antigenic differentiation by production of monoclonal antibodies after concentrating of the supernatants of CCs, infected with *PGPV* and *CNPV* has been done. For this aims, homologous recombination by using of plasmid vectors, containing suitable promoter, and a segment from the *FWPV* DNA, coding non-essential region of the virus genome has been used [43]. It has been inserted the gene, coding the enzyme β-galactosidase from *E. coli* in the produced recombinant *APV* (*rAPV*) [44]. *rFWPVs*, expressing genes for the haemagglutinin (HA) of the avian influenza virus have been constructed [20-22]. Later *rFWPVs* have been used for first time for *in ovo*-inoculation [23]. In these cases, the expression of haemagglutinin-neuraminidase (HA-NA) and of the fusion glycoprotein (F) in CEFs has been proved by immunohistochemical assay [23, 24].

In 2001, a stable *rFWPV*, expressing the C-terminal region (consisting of 119 aminoacid remainders) of the nucleocapside protein (N) of the IBV, strain Ch3, has been constructed by insertion of a coding sequence on *tk* gene region of the *FWPV* by homologue recombination [25]. The inserted nucleocapside protein has been expressed in CEFs under the control of the vaccinia virus promoter *P7.5*. For genetic differentiation of *APVs* polymerase chain reaction (PCR) has been added. In this way, a gene, coding protein with molecular weight 39 kDa from adapted on CCs *TKPV*, has been identified. Before that the propagation of *FWPV* and the provoked pathological changes in CEFs has been observed. These changes have been observed at the third day after the inoculation of the cultures, and they have been expressed mainly in vacuolization of the cytoplasm, rounding of the cells and necrosis. Besides that, a presence of cytoplasmic eosinophilic inclusions has been demonstrated [25–50,53-55]. On the fourth day post inoculation (p. i.) yellow and greenish fluorescence in staining with acridine orange has been observed, and on the sixth day p. i. – only green fluorescence of the cytoplasmic inclusions. These results have proved the presence of virus DNA in the cytoplasm of the infected cells. Besides that, on the sixth day p. i. the authors established that the nuclei and the cytoplasmic inclusions in the cells stained in pink (a positive reaction by the Fewlgen test), which was also an indication that these inclusions were composed by virus DNA. The cultivation of *APVs* in CCs could be also used for another diagnostic methods: immunodiffusion in agarose gel, immunofluorescence, virus neutralisation, passive haemagglutination, enzyme-linked immunosorbent assay (ELISA), immunoperoxidase reaction.

In the last years if the 20th century have been made experiments for adaptation and propagation of APVs in mammalian CCs [56-58]. The experiments for propagation of *FWPVs* in monkey (Vero and CV-1) and human (HeLa and MRC-5) permanent cell lines (CLs) after their previous cultivation on CEFs, have shown that a

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probability for production of mature virions exists [56]. Later similar experiments were done with the CLs MDBK and EBTr, derived from embrional bovine kidney and trachea, respectively [57,58]. The authors have observed a slow CPE, which was also an indication that the *APVs* replicate productively in a reduced number of infected mammalian cells. Results, which demonstrated the activity of expression of the inserted from *Esherichia coli* gene for the enzyme β-galactosidase in CEFs, as well as in the Vero CL, infected with such rAPVs, have been received. Besides that, it has been shown that *rFWPVs* and recombinant *canarypox viruses* (*rCNPVs*), which contained genes, coding some glycoproteins of the rabies virus and the measles virus, respectively, under the control of *vaccinia virus H6*-promoter, could express these genes in mammalian cells [55]. Similar results have been received about the activity of expression of the same enzyme gene in CCs of the Vero CL and in CEFs for studying of the expression of the late genes of the *FWPV* in the used CLs [56]. *rFWPVs*, expressing genes of some herpes viruses in mammals in CCs of the monkey CL Vero and of the human MRC-5 CL, have been produced. Although in inoculation of mammals and mammalian CLs with production of *APVs* of mature virus particles is not established or the level of the expression of late genes has been very low, the expression of early genes has been proven. In this way, a safety, which has been proven as an advantage in comparison with the using of virus recombinants, based on viruses, which has provoked productive infections, has been ensured. In contrast to *vaccinia virus*, *CNPV*, as well as all other *APVs*, have replicated productively in mammalian cells, without production of infectious virus particles. Because of these reasons, CCs of mammalian CLs have been used for replication of *rFWPV*, expressing the gene for HA of the human influenza virus. At the same time the authors have studied the effect of the co-expressed cytokines IL-2 and γ-IFN. Later, permanent mammalian CLs were applied for production of *rFWPVs*, expressing genes for the carcinoembrional antigen (CEA) and for the prostate-specific antigen (PSA), respectively [59]. Parallel with these genes, such for co-stimulating molecules have also been expressed.

Recombinant Vaccines, Based on *Avian Pox Viruses*

Vaccines, based on *poxvirus* vectors, have been found to leave a possibility for studying of the induction of the immune response [16-55,59-79]. Their applications have also been examined in tumor immunotherapy and gene therapy. By deletions of specific virus genes there have been received vaccines *NYVAC*, derived from attenuated *vaccinia virus*, Copenhagen strain, *TROVAC*, received from attenuated *FWPV*, and *ALVAC*, derived from a vaccine strain of *CNPV*. According to much literature data the recombinants, based on the *CNPV* genome are more effective in comparison with these, based on the FWPV. It has been considered that the creating of one of these recombinants is the first phase of the developing of such vaccine in humans [37]. In this case, in *CNPV* genome a gene, coding glycoprotein of the rabies virus has been inserted. The ability of recombinants, based on the *CNPV* genome, by induction of specific immunity against rabies in cats and dogs, fever in dogs, leucosis in cats and against the equine influenza virus, has been studied [32-36].

In humans, recombinant vaccines, based on the *CNPV* genome, which have expressed antigens of rabies, measles, Japanese encephalitis virus, as well as of some strains of the human influenza virus, have turned out harmless and high immunogenic. Besides that, recombinant *canarypoxviruses* (*rCNPVs*), containing sequences from HIV-1-genome, have been proven as promising vaccine "candidates". *rAPVs* have also been applied for treating of some types of solid tumors in humans. Hence, infection with *rAPVs* could stimulates the cell and the humoral immune response against HIV, as well as against some tumor-associated antigens.

One of the most widely used expression systems in the molecular biology has been characterized *vaccinia virus* [17,41-74]. However, in vaccination with *vaccinia-virus*-based recombinant vaccines – for example a danger for risk hosts, some problems have arised. The possible solution to this might be the use of *APV* vectors, for example, *FWPV* and *CNPV* for such aims in view of the next causes: abortive replication in mammalian cells; undergoing of the abortive replication in a small number of mammalian cells; high level of expression of foreign proteins; no danger of pathogenesis, as well as no natural immunity in humans [18,21, 26-28,30-38].

For expression of foreign genes, an integration of the foreign DNA in the *poxvirus* genome by homologous recombination or by a direct molecular cloning has been found to be necessary [39]. In both cases, *plasmid* vector constructions, containing the desired genes and at least one marker gene, have been used [40]. The necessity both types of genes to be controlled by suitable promoters has also been proven. For facilitation of this technique, a modified *vaccinia virus* genome, which has been proven as able to admit an direct insertion of DNA-fragments in the region of the *vaccinia virus* promoter without no forward steps of cloning, has been constructed [41]. The inserted gene has been amplified by polymerase chain reaction

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(PCR). Oligonucleotide primers, ensuring *Sfi*-site on the 5/-end, and *Rsf*-site on the 3/-end of the derived PCR-product have also been used [39-41]. In the digesting with the respective restrictases, the obtained PCR-product has been found to be connected with a synthetic promoter sequences in virus DNA-genome. However, intermediate plasmid constructions in this method haven't been necessary. Techniques, used for construction of recombinants, based on *vaccinia virus*, might be easy applied for construction of recombinant vaccines, based on *APVs* [39- 46]. The possibility for insertion of some genes in the viral genome of the *FWPV* could allow the construction of polyvalent vaccines, as well as of vaccines, containing modificators of the immune response, as, for example, genes for lymphokines. For insertion of foreign genes in *FWPV* genome, *tk*, terminal repeats, as well as a site, homologous of this of the *vaccinia virus*, have been used. The homology between *vaccinia virus* and *FWPV tk* has been confirmed both at level nucleotides and aminoacides [40,41].

Identification, cloning and mapping of main gene, expressed in the early and late stages of infection with *FWPV*, has also been realized. The necessity of the early function of this gene for construction of recombinant *fowlpox viruses* (*rFWPVs*) has been established. A *poxvirus* promoter for ensuring the expression of the inserted vaccine antigen, has also been proven to be necessary. It has been suggested that the strength of the used promoter could be an important condition in respect to the quality of the expressed vaccine antigen(s) and the inserted fragments could cause attenuation in some regions of the derived *rFWPV*. As one of the methods for refining of the recombinant vaccines, has been proven the using of natural or synthetic immunomodulating agents, some of which have been found to enhance the protection of the host, but other could repress the natural and the acquired immunity [30-39]. As potential immunomodulating agents have been used cytokines (interferons and interleukines), because they are natural proteins, which have been found to be included in regulation of the immune response, as well as in the excitement of the live organisms. It has also been shown that the co-expression of the gene for IL-6 with gene for HA of the influenza virus in *FWPV*-vector stimulates the humoral immune response in mice. In this way, *rFWPVs*, expressing HA of the avian and human influenza virus, respectively, have been constructed. An additional possibility of vaccines, based on the *FWPV* and/or other *APV* vectors, has been the suggestion that these viruses could be suitable for vaccination of non-avian species. It has been established that the inoculation of mammalian cells with recombinants, based on *APVs*, results in expression of the foreign gene, and the inoculation of mammals with such recombinants – in induction of protective immunity.

Later, a strategy, based on immunization with DNA-plasmids and *rFWPV*, both coding common vaccine antigen has been developed [52-55]. This method has been characterized as one of the most reliable vaccinations against HIV-1, and increased levels of T-cytotoxic lymphocytes (T-CTL) and anti-HIV-antibodies have been established. In this way, plasmid DNA and *rFWPV* have been between the most promising and most sure vaccine "candidates" against this virus strain, but the immunity, induced only by one of the vaccine components, might not be enough for ensuring of permanent protection against the infection with HIV-1.

Therapeutic Potential of Gene-Engineered Stem and Progenitor Cells

The evidences purporting the differentiation of the stem/progenitor cells into different cell lineages and evaluate the utility of these cells as cellular vectors for treating diseases, have been reviewed [80-83]. Because these gene constructs haven't shown cytotoxicity in normal human cells, their therapeutic efficacy has been suggested by the results obtained. For example, in testing of the role in survival by intratumoral treatment of tumor-bearing experimental animals with HSV, expression of IL-4 has been found to prolong their survival, whereas expression of IL-10 – to its reduction. To assess the strategy of combining oncolytic herpes simplex virus (HSV) therapy with immunomodulatory therapy as treatment for experimental colon cancer, the oncolytic *HSV* recombinant *NV1023* and the IL-12-secreting oncolytic *NV1042* virus have been evaluated *in vivo* and *in vitro* with respect to antitumor efficacy, and both *NV1023* and *NV1042* have had the potential to kill colon cancer cells at higher doses [79,84]. Optimal survival of isolated plasma cells has been found to require stimulation by a combination of factors acting synergistically.

These results have strongly supported the concept that plasma cell survival depends on niches in which a combination of specific signals, including IL-5, IL-6, stromal cell-derived factor-1α, TNF-α, as well as ligands for CD44, provides an environment required to mediate plasma cell longevity. These findings could have implications in the development of specific second-generation cancer immunotherapy protocols. Immunization with several *canarypox virus* recombinants

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has protected BALB/c mice from a challenge with an isogenic and highly tumorigenic mouse fibroblast tumor cell line, expressing high levels of mutant p53 [35- 38]. On the other hand, in these experiments has been demonstrated that normal human mammary epithelial cells can be immortalized by transfection with virus DNA from human papilloma viruse strains 16 and 18 (HPV-16 and HPV-18), although these viruses have not been associated with breast cancer [69,70]. The effective immortalization, as well as other pre-malignant changes, induced by transfection with HPV, have been found to be accompanied by chromosome changes, that may contribute to the partially transformed phenotypes. However, none of the cloned or pooled transfectants have been tumorigenic in the nude mouse assay. Immortalization has been experimentally separable from tumor-forming ability.

Lentiviruses have also been characterized as potentially advantageous compared to oncoretroviruses as gene transfer agents, as well as efficient tools for gene transfer stem cells, mainly because of their ability to infect non-dividing cells [85-100]. These of them, which contain transgene *LacZ*, have been found to achieve detectable β-galactosidase activity in rat arteries, albeit at a lower level compared with adenovirus vectors, mainly due to the lower concentration of *lentivirus* vector preparations [52-55,85-100].

It has also been suggested that by *lentivirus* vectors can be obtained using robust and tightly regulated knockdown of gene expression. In allogeneic stem cell transplantation, MSCs may be used for hematopoiesis enhancement [101]. It has also been proposed that resting HSCs may be transduced by *lentivirus*-based, but not *MuLV*-vectors, and these results have maintained their primitive phenotype, pluripotentiality, as well as, transgene expression *in vitro* [99,100]. These findings have indicated a predictive value of gene transfer measurements to such LTC-IC for the design of clinical gene therapy protocols. Besides that, *retrovirus*-mediated transduction of human telomerase reverse transcriptase (hTERT) in human dermal microvascular embryonic cells (HDMECs) *in vitro* has resulted in cell lines that form microvascular structures after subcutaneous implantation in severe combined immunodeficiency (SCID) mice [102]. In addition, human bronchial, corneal and skin cells, expressing *hTERT*, have been found to be used to form organotypic cultures (bioengineered tissues) that express differentiation-specific proteins, demonstrating that *hTERT* by itself does not alter normal physiology. The production of *hTERT*-engineered tissues has offered the possibility of producing tissues to treat a variety of chronic diseases and age-related medical conditions that are due to telomere-based replicative senescence [2-7,85-100].

Telomere shortening has been found to inhibit mobilization of stem cells out of their niche, impaired hair growth and resulted in suppression of stem cell proliferative capacity *in vitro*. In contrast, overexpression of gene *TERT* in the absence of changes in telomere length has promoted stem cell mobilization, hair growth and stem cell proliferation *in vitro*. The effects of telomeres and telomerase on stem cell biology has been found to anticipate their role in cancer and aging [2-7,85-100]. Restoring of the telomerase activity as a putative therapeutic strategy has necessitated further study to elucidate the intricacies linking genetic and epigenetic modulations of gene *hTERT*. It has been shown that, when lesion repair is imperfect, immortal strand co-segregation leads to better preservation of the stem cell lineage than random chromosomal segregation. On the other hand, *HIV*-based vectors have been shown as promising tools for studies on gene functions in primary human B-lymphocytes and myeloma cells for the purposes of research, as well as the development of gene therapies [103,104]. Successful use of this virus strain in treatment of tumors both directly and by tumor-targeted gene therapy vector has been obtained in transduction of human gene *IFN-con1*. Differentiation of engrafting HSCs to peripheral blood cells has been established to not be necessarily associated with a significant suppression of retroviral gene expression, which has accentuated the importance of empirically testing *retrovirus* vectors to determine lasting

in vivo-expression [105 -109]. As a useful tool for *in vitro*-tumor cell modeling and anticancer drug screening, as well as for cancer gene therapy, has been found alginate-poly-L-lysine-alginate microcapsule because of its influence on the proliferation, viability and metabolism of human cells, including anchorage-dependent MCF-7 breast cancer cells and primary fibroblasts, anchorage-independent leukemia cells K562, as well as cells in conventional culture have been used as a control [109]. Cells that have undergone immortalization via a crisis period of transformation by chemicals or viruses, as well as malignant cell lines in general, have had an ability to divide indefinitely. Mice, immunized with extracellular domain of oncogen *Neu* have been protected against a subsequent inoculation of MCNeuA cells, indicating that this cell line will be useful for evaluating cancer vaccine strategies. It has been demonstrated that primary

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myoepithelial cells from normal breast reduce breast cancer cell invasion and that this is mediated via modulation of both tumor cell and fibroblast function [109]. These results have emphasized the importance of the myoepithelial cell in controlling the breast microenvironment and focuses on the potential significance of the loss of this population with disease progression. According to other results, *HIV-1*-based vectors have appeared to be very efficient for delivering and expressing transgenes in MSCs [110-112]. *HIV-1*-based vectors in the context of MSCs may have clinical applications once the safety issues that are inherent to this vector system have been resolved.

Specific anti-gp100 T-cells, induced by *in vivo*-vaccination of experimental mice with recombinant *Ad2CMV-gp100*, have been found to be responsible for their protection. For example, in infection of malignant cells A549 with gene construct *Ad-hEndo*, in the infected tumor cells has been detected recombinant endostatin protein, and its inhibitory effect on endothelial cells growth has been shown.

Conclusion

It has been indicated that CCs from primary embryo cells, cell clones and CLs are convenient systems, which could be used for cultivation of viruses and for diagnostic aims, as well as for isolation and detection of viruses. Besides that, the system “cell culture-viruses” could be applied for different medical and biological aims. In the case, as an appropriate example has been characterized the application of cultivated avian pox viruses for production of rAPVs, expressing different antigens and hence – the possibility for using of such recombinants for therapy of different diseases by activation of the immune system. These recombinants could be used for such aims for birds, if the “parent” virus has been cultivated on avian CCs, and for mammals, including people, if the “parent” virus has been cultivated on mammalian CCs, respectively. APVs have been found to possess some advantages in comparison with vaccinia virus because of the abortive replication in mammalian cells and, hence, the possibility of less danger of pathogenesis. In in vitro-conditions, however, these viruses have been able to undergo the abortive replication in mammalian cells, and, in this way, they could be used for production for the pointed above biotechnological aims. Studies on the stem/progenitor cell biology are focused on their abilities for self-renewal and differentiation. These properties make them strong candidates for delivering of genes and restoring organ systems function, which have been established to be included in these processes. This idea has given rise to the notion that many aspects of the normal aging could primarily reflect limitations in DNA-repair and telomeric-maintenance pathways in the stem/progenitor cells of the soma. As a particular aim has been found the possibility for use of hematopoietic stem cells and/or of mesenchimal stem cells in various forms of cellular therapies, as well as genetic tools that can be used for a better understanding of the mechanisms leading to repair and regeneration of damaged and/or transformed cells. In this aspect, technique for preparing of cell vectors by transduction of genes in stem/progenitor cells, by virus gene constructions, has been established as a perspective for new therapeutic strategies.

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