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In vitro-differentiation of Embryonic Mammalian Cells as a Material Base for Gene-engineering Manipulations

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Summary: In *in vitro*-cultivation and infection of cells from the mammalian cell line EBTr, derived from embryonic bovine trachea, with low initial titers of the DNA vaccine avian pox viral strains FK and Dessau, pathological changes in the inoculated cells were not observed or they were slight. On the other hand, differences between the cells from this line were observed independently of presence or absence of virus infection and they were probably due to their further differentiation. These results indicated that, so applied, the used heterologous for mammals and/or mammalian cells vaccine virus strains, are probably non-pathogenic for them. In agreement with literature data, such safe viral strains might be used for transduction of genes in *in vitro*- and/or *ex vivo*-cultivated stem/progenitor cells, by obtained on their bases virus gene constructs.

Keywords: Stem/progenitor Cells, Cell Differentiation, Cancer, Vaccine Viral Strains, Viral Gene Constructs.

1. INTRODUCTION

The most important approaches currently utilizing stem and/or progenitor cells are gene therapy and tissue engineering [3, 4]. The evidence purporting the differentiation of their into different cell lineages and evaluate the utility of these cells as cellular vectors for treating malignant diseases, has been reviewed [5-8, 12, 13, 15].

Many literature data have suggested the potential of virus-based vectors for the development of various therapeutic strategies [5-8, 12]. The different mechanisms of application of gene constructs *Ad-IGFBP3* and *SCH66336*, based on adenoviral genomes, on cancer cells, have been found to increase their apoptosis *in vitro* and *in vivo*, and at the same time – to decrease the expression of genes, which has been proven as activators of the process of programmed cell dead, in these cells. Besides that, these gene constructs haven't induced detectable cytotoxicity in normal cells [5]. These results

have been supported by the date, according which gene construct *Ad-dnp85alpha*, also based adenovirus genome, has significantly inhibited proliferation of cell lines, derived from human non-small cell lung cancer (NSCLC) [5-8, 12].

In this aspect, the aim of the present work was to prove the safety for mammals of heterologous for them vaccine viral strains, and in this way – a possibility for preparing on their basis of necessary for transduction of genes in undifferentiated stem/progenitor cells from mammals, gene constructs.

2. MATERIALS AND METHODS

Cells from the mammalian cell line EBTr, derived from embryonic bovine trachea, have been used [1, 11]. Cultivation was performed in a humidified 5% CO_2/95% air incubator at 37^{o}C. Cells (in initial volume 22x10^5 on 1 ml cultural fluid) were routinely grown in a growth medium, a combination of Minimal Eagle's Medium (MEM) (Sigma) and Dulbecco's Modification of MEM (DMEM) (Sigma), in ratio 1:1, supplemented with 5% normal bovine serum (Sigma), and a respective antibiotics in volumes (100 IU/ml Penicillin and 100 μg/ml Streptomycin) (Sigma) were added. So cultivated, cells were inoculated after 24 hours in 24-well plates (24 Nunclon; Space Sever Flow Lab.; Linbro), as semiconfluent monolayers, with the avian DNA vaccine pox viral strains FK (fowl) and Dessau (pigeon). The used initial infectious virus titers (equivalent of reciprocal values of the respective dilutions of the initial virus suspensions), were measured in 50% cell-cultural infectious doses on 1 ml cultural fluid ($CCID_{50}$/ml). After absorption for 45 minutes at room temperature, the monolayers were washed three times with 1 ml on a well of phosphate buffered solution (PBS, pH 7.2) and 1ml on a well of supporting medium, a combination of MEM (Sigma) and DMEM (Sigma) in the ratio 1:1, supplemented with 2% normal bovine serum (Sigma), was added. Controls were always included and similarly processed. These preparations were observed under invert microscope Televal at 24-hour intervals.

3. RESULTS AND DISCUSSION

In consequent cultivation of vaccine avian pox virus strains in cell cultures from the embryonic line EBTr, in every passage a decrease

of the in the pathological changes in the inoculated cells has been observed (Table 1). This result was probably due to attenuation in their consequent passages, and it was in agreement with the literature data [9, 14].

Table 1. Degree of pathological changes in cells from embryonic mammalian line EBTr after viral inoculation with non-diluted virus suspension (10^0 $CCID_{50}$/ml) of the vaccine avian pox viral strains.

Vaccine virus strains	Number of passages on embryonic mammalian cell line EBTr	Degree of pathological changes in the infected cells after
FK	1	3 - 4+
Dessau	1	1+
FK	2	3 - 4+
Dessau	2	1+
FK	3	3 - 4+
Dessau	3	1+
FK	4	4+
Dessau	4	+
FK	5	4+
Dessau	5	1 - 2+
FK	6	4+
Dessau	6	1 - 2+

In using of comparatively low initial infectious titers for both strains – 10^3 $CCID_{50}$/ml (comparatively high delusions, respectively – 10^{-3} $CCID_{50}$/ml) of viral suspensions (Table 2), pathological changes in the infected cells weren't observed or they were slight.

The observed lacking or low pathological changes in the inoculated cells, in using of low initial viral titers, supposed that, the used heterologous for mammals and/or for mammalian cells virus strains are probably non-pathogenic for them (Table 3).

The observed changes between the cells from the cell line were observed independently of presence or absence of virus infection and were probably due to further cell differentiation [10, 11]. The results obtained confirmed the literature data for the safety of used for

transduction of genes in stem cells viral gene constructs [2, 5-8, 10, 12, 13, 15].

Table 2. Initial infectious titers of the used virus strains on heterogenic for them cells of the embryonic mammalian cell line EBTr, respectively on 72nd, 96th and 120th hours after viral inoculation of the cells, measured in 50% cell-culture infectious doses on 1 ml cultural fluid ($CCID_{50}$/ml). Only the values on the last, 120th hour post inoculation are presented in the paper – $10^3 CCID_{50}$/ml, for both strains.

Hours after inoculation	Initial infectious titer of vaccine virus strain FK ($CCID_{50}$/ml)	Initial infectious titer of vaccine virus strain Dessau ($CCID_{50}$/ml)
72	10^3	10^2
96	10^3	10^3
120	10^3	10^3

Table 3. Titration of vaccine avian pox virus strains FK and Dessau on the cells from line EBTr and degree of caused by them pathologic changes (10-fold dilutions of the initial dilution 10^3 $CCID_{50}$/ml for both strains).

		Hours post viral inoculation							
Virus strains	Dilutions ($CCID_{50}$/ml)	24	48	72	96	120	144	168	192
FK	10^0	-	-	-	-	-	-	-	-
Dessau	10^0	-	-	-	-	-	-	-	-
FK	10^1	-	-	+/-	+/-	+/-	+/-	+/-	+/-
Dessau	10^1	-	-	-	-	-	-	-	-
FK	10^2	-	-	+/-	+/-	+/-	+/-	+/-	+/-
Dessau	10^2	-	-	-	-	-	-	-	-
FK	10^3	-	-	+/-	+/-	+/-	+/-	+/-	+/-
Dessau	10^3	-	-	-	-	-	-	-	-
FK	10^4	-	-	+/-	+/-	+/-	+/-	+/-	+/-
Dessau	10^4	-	-	-	-	-	-	-	-
FK	10^5	-	-	+/-	+/-	+/-	+/-	+/-	+/-
Dessau	10^5	-	-	-	-	-	-	-	-

4. CONCLUSION

The observed differences between the cells from the used embryonic mammalian cell line were probably due to their further differentiation. Hence, applied on the described way, the used heterologous for mammals and/or mammalian cells vaccine virus strains, are probably non-pathogenic for them. Such safe viral strains could be convenient as an initial material for possible future applications in gene-engineering manipulations, like transduction of genes in *in vitro*- and/or *ex vivo*-cultivated stem/progenitor cells from mammals, by prepared on their basis gene constructs. For both aims, future studies are necessary.

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REFERENCES

1. Kniazeff A., Nelson-Rees W., N. Darby, *Experta medica*, 1965, 19, Section 1.
2. Koch P., H. Siemen, A. Biegler, J. Itskovits-Eldor, O. Brüstle, Transduction of Human Embryonic Stem Cells by Ecotropic Retroviral Vectors, *Nucleic Acids Res.*, 2006, 34, e120.
3. Lansdorp P. M., Role of Telomerase in Hematopoietic Stem Cells, *Ann. N. Y. Acad. Sci.*, 2005, 1044, 220-227.
4. Lansdorp P. M., Telomere Length and Proliferation Potential of Hematopoietic Stem Cells, *J. Cell. Sci.*, 1995, 108, 1-6.
5. Li L., J. L. Huang, Q. C. Liu, et al., Endostatin Gene Therapy for Liver Cancer by a Recombinant Adenovirus Delivery, *World J. Gastroenterol.*, 2004, 10, 1867-1871.
6. Li L., P. H. Wu, J. L. Huang, et al., Inhibitive Effects of Recombinant Adenovirus-mediated Human Endostatin on the Growth of Human Hepatocellular Carcinoma Xenograft in Nude Mice, *Zhonghua Gan Zang Bing Za Zhi*, 2003, 11, 542-545.
7. Li L., R. Y. Liu, J. L. Huang, et al., Adenovirus-mediated Intratumoral Delivery of the Human Endostatin Gene Inhibits Tumor Growth in Nasopharyngeal Carcinoma, *Int. J. Cancer*, 2006, 118, 2064-2071.

8. Li X. P., C. Y. Li, X. Li, et al., Inhibition of Human Nasopharyngeal Carcinoma Growth and Metastasis in Mice by Adenovirus-associated Virus-mediated Expression of Human Endostatin, *Mol. Cancer Ther.*, 2006, 5, 1290-1298.
9. Mockett A., A. Deuter, D. Southee Fowlpox Vaccination: Routes of Inoculation and Pathological Effects, *Avian Pathology*, 1990, 19, 613-625.
10. Rattmann I., V. Kleff, A. Feldmann, C. Ludwig, U. R. Sorg, B. Opalka, T. Moritz, M. Flasshove, Reliable Generation of Stable High Titer Producer Cell Lines for Gene Therapy, *Intervirology*, 2007, 50, 197-203.
11. Sainova I. V., A. I. Kril, K. B. Simeonov, T. P. Popova, I. G. Ivanov, Investigation of the Morphology of the Cell Clones, Derived from the EBTr Cell Line and Their Susceptibility to Vaccine Avian Poxvirus Strains FK and Dessau, *J. Virol. Meth.*, 2005, 124, 37-40.
12. Schenelmann S., L. M. Ogilvie, D. Hedley, F. Friedlos, J. Martin, I. Scandol, P. Chen, R. Marais, C. J. Springer, Suicide Gene Therapy of Human Colon Carcinoma Xenografts using an Armed Oncolytic Adenovirus Expressing Carboxypeptidase g2, *Can. Res.*, 2007, 67, 4949-4955.
13. Seppen J., S. C. Barry, J. H. Klinkenspoor, L. J. Katen, S. P. Lee, J. V. Garcia, W. R. A. Osborne, Apical Gene Transfer into Quiescent Human and Canine Polarized Intestinal Epithelial Cells by Lentivirus Vectors, *J. Virol.*, 2000, 74, 7642-7645.
14. Somogyi P., J. Frazier, M. Skinner, Fowlpox Virus Host Range Restriction: Gene Expression, DNA Replication, and Morphogenesis in nonpermissive Mammalian Cells, *Virology*, 1993, 197, 439-444.
15. Xiong C., D. Q. Tang, C. Q. Xie, L. Zhang, K. F. Xu, W. E. Thompson, W. Chou, G. H. Gibbons, L. J. Chang, L. J. Yang, Y. E. Chen, Stem Cells and Development: Genetic Engineering of Human Embryonic Stem Cells with Lentiviral Vectors, *Neural Stem Cells Compendium*, 2005, 14, 367-377.