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Research Article

Experimental *In Vitro*-Stem Cell Models for Balanced Activity of Oncogenes and Tumor-Suppressor Genes from Mouse and Human

Iskra Sainova[1], Ilina Vavrek[1], Velichka Pavlova[1], Teodora Daneva[2], Ivan Iliev[1], Lilija Yossifova[1], Elena Gardeva[1], Elena Nikolova[1]

[1]Department of Experimental Morphology, Institute of Experimental Morphology, Pathology and Anthropology with Museum to Bulgarian Academy of Sciences, 1113 Sofia, Bulgaria

[2]Department of Immunobiology of Reproduction, Institute of Biology and Immunology of Reproduction to Bulgarian Academy of Sciences, 1113 Sofia, Bulgaria

ABSTRACT

Taking in consideration many literature data, according which coordinated oncogenes and tumor-suppressor genes functions is of most importance. In this connection, the main idea was balanced activity of oncogenes and tumor-suppressor genes in vitro in laboratory-cultivated normal cells be achieved. Additional copy of oncogene was inserted Dcn1 in mouse embryonic stem cells (mESCs) by their transfection with appropriate recombinant DNA-constructs. On the other hand, in cultivation of separate sub-populations of non-transfected mESCs in the presence of Doxicyclin, suppressed cell proliferation and increased myeloid cell differentiation was observed, probably by activation of genes from STAT-family. After in vitro-co-cultivation of sub-populations of transfected mESCs, containing additionally-inserted copy of oncogene Dcn1 with sub-populations of non-transfected mESCs, treated with Doxicyclin, the results obtained have indicated the safety and preserved normal/non-tumorigenic cell character of the so derived stem cells in vitro, despite of the presence of additional oncogene copy. These data were confirmed by results from similar experiments with analogical human normal trophoblast cells, immortalized by transfection with SV40 recombinant gene vector.

Key words: *stem cells, oncogenes, tumor-suppressor genes, cell transfection.*

INTRODUCTION

Coordinated oncogenes and tumor-suppressor genes activity has been found to be of most importance [11,24,28-30]. In this connection, Janus-kinases (JAKs)–signal transducers and activators of transcription (STATs) pathway, has been shown to be a common signalling pathway, used by many cytokines [5,9,23]. In this aspect, cytokine-activated Janus-kinases (JAKs)–signal transducers and activators of transcription (STATs) pathway has shown an important role in the control of immune responses, and dysregulation of it has been associated with various immune disorders. Signalling strength, kinetics and specificity of this pathway have been shown to be modulated at many levels by distinct regulatory proteins.

Studies on the biology of the stem cells are often focused on their self-renewal and differentiation [1,8,15,19,21,24-26,28,29]. A lot of variations of the DNA-repair efficiency of has been established to vary greatly among different stem cell types [8,15,19]. This high self-renewal potential of the stem 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 [19,24,28,29]. This understanding could be applied toward the ultimate goal of using stem 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 both *in vivo* and *in vitro* [25,28]. The properties of "malignant stem cells", have outlined initial therapeutic strategies against them [26,29].

As the most important approaches, currently utilizing stem cells, both gene therapy and tissue engineering have been determined [2-4,6,10,27]. Both have been found to exploit the current knowledge in molecular biology and biomaterial science in order to direct stem cells *in vitro*- and/or *in vivo*-differentiation to desired lineages and tissues. In this aspect, widely studied is the ability for laboratory cultivation of viruses in cell cultures, with the aim for development of both viral recombinants for malignant immunotherapy and of products for therapy of these disorders. As such

tools can be used both DNA- and RNA-viruses [2,3,6,27], as well as bacterial plasmids [10] and yeasts [4].

In this connection, the main goal was connected with a design of maximally safe experimental *in vitro*-model for providing, on the one hand, of active tumor-suppressor gene for prevention of eventual malignant transformations, and, on the other hand, of active oncogene for prevention of early aging and death. For comparison, normal cells from human trophoblast cell line HTR8/SVneo, immortalized by transfection with *SV40* recombinant gene vector [5,13,14,17,22,27], were used.

MATERIALS AND METHODS

Stem cells, isolated from mouse Balb/c embryos, were cultivated for 48 – 72 hours on previously formed monolayers of feeder primary MEFs after their previously treatment by Mitomycin-c (mm-c) (Sigma-Aldrich) and/or 3T3 fibroblasts. After tripsinization, they were transfected by electroporation (5 X 10^6 cells/ml). For this aim, recombinant DNA-genome from *adeno-associated virus* (*AAV*) (*Parvoviridae*) [6], containing promoter for gene, coding Elongation Factor 1-alpha (*EF1-α*); gene *Dcn1*, isolated from 3T3 fibroblasts of laboratory mice Balb/c, as well as gene for neomycin resistance, isolated from bacterial DNA-plasmid, are used. For this goal, electroporator for cell transfection (BioRad) was used. Separate sub-populations of non-transfected mESCs were cultivated in the presence of 2 μg/ml Doxicyclin (Sigma-Aldrich) for suppression of cell proliferation and eventual stimulation of myeloid cell differentiation by activation of genes from *STAT*-family [18]. On the other hand, cell cultures of the derived from human cervical carcinoma cell line Hela were also prepared. All cells were incubated at 37^0C in incubator with 5% CO_2 and 95% air humidification, in Dulbecco's Modified Minimal Essential Medium (DMEM) (Sigma-Aldrich), supplemented with 10% Fetal Calf Serum (FCS) (Sigma-Aldrich), 100 U/ml penicillin (Sigma-Aldrich) and 100 μg/ml streptomycin (Sigma-Aldrich), and observed by inverted light microscope (Leica).

After tripsinization of the transfected cells and their consequent treatment with mixture of phenol-chlorophorm-isoamil alcohol (PCI) (Sigma-Aldrich), the so isolated nuclear material was treated with lysis buffer (Sigma-Aldrich) for isolation of genomic DNA. The last was subjected on Polymerase Chain Reaction (PCR) of previously isolated nuclear DNA of them and its consequent 1% agarose gel (Sigma-Aldrich) electrophorhesis, in the presence of DNA-primers against the inserted DNA-fragment (Sigma-Aldrich), mixture of the four types deoxy-nucleosid-tri-phosphates (dNTP - Sigma-Aldrich), enzyme Taq-polymerase (Sigma-Aldrich).

Fixed light microscopic preparations were prepared by their consequent fixation by treatment with 95% ethanol (Sigma-Aldrich) or paraphormaldehyde (Sigma-Aldrich), washing with 1:9 diluted PBS (Sigma-Aldrich) and Giemsa-staining (Sigma-Aldrich).

RESULTS

In our experiments 9 transfected by electroporation cell clones were received and derived (Figure 1). According to the genomic assays results, 2 of the cell clones derived were positive on the additionally inserted copy of the oncogene *Dcn1* and the other 7 cell clones - negative on it (Figure 1 – a). These results were confirmed by the data, obtained by electrophorhesis of the used recombinant DNA-constructs in the same conditions (Figure 1 – b).

Decreased cell proliferation level on the one hand and active myeloid differentiation on the other was established in cultivation of cell sub-populations in the presence of Doxicyclin (Figure 2).

DISCUSSION

According to different literature findings genetic interactions between oncogene and tumor-suppressor genes, as well as influence of the protein product on the one or two genes on the structure and functions of the other of both genes [14,17,22,29,30], could be possible. The observed effects of early myeloid differentiation and suppression on the cell proliferation in the presence of Doxicyclin could be explained with the described in many literature sources activation effect of this substance on the tumor-suppressor genes of *STAT*-family (Figure 2) [19,24]. Similar types of correlations of gene *p53* has recently been proven with gene *NUMB*, which has been characterized as a cell fate determinant because of its role in the asymmetric cell division in the mitosis process, as well as between gene *Oct4*, which is known as regulator of the processes of stem cell self-renewal and differentiation and gene variation *Cdk2ap1*, by a mechanism of *Oct2/4* promoter methylation [7].

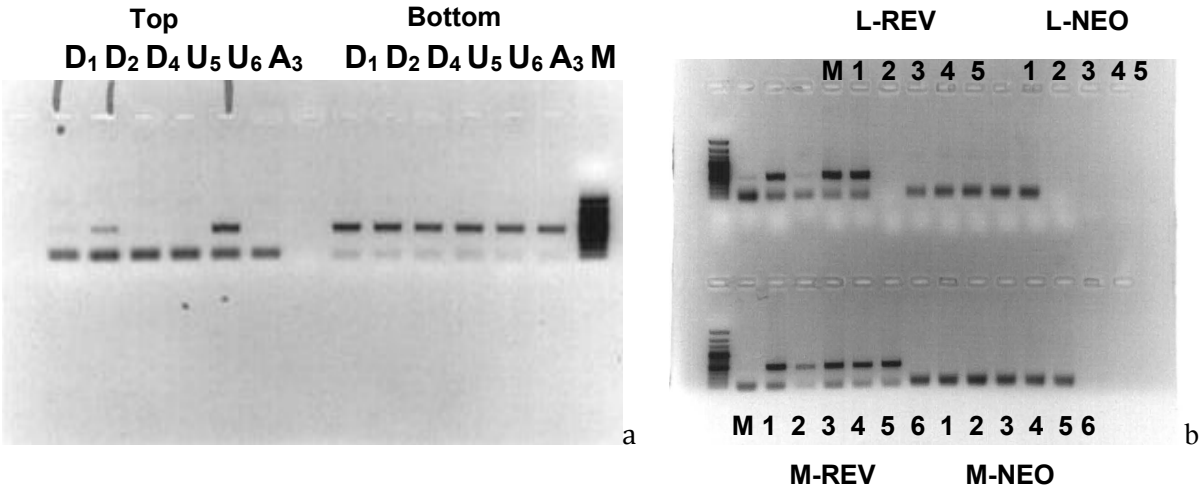

Figure 1. Agarose gel electrophorhesis for prove of the presence and/or the absence of additionally-inserted copy of the oncogene *Dcn1* in cell clones, derived from transfected by electroporation *in vitro*-cultivated mESCs (a) and in the used for cell transfection recombinant gene constructs (b).

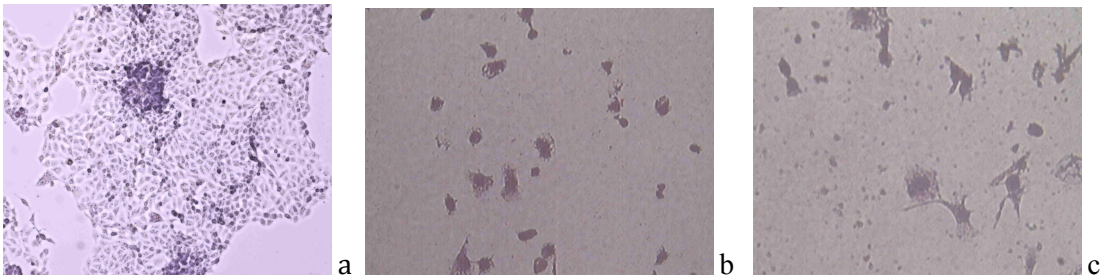

Figure 2. *In vitro*-cultivated sub-populations of non-transfected mESCs: in the absence of additional external factors of differentiation (a); decreased levels of cell *in vitro*-proliferation and activated *in vitro*-differentiation, in particular in myeloid precursor cells, by activation of the tumor-suppressor genes from *STAT*-family by cultivation in the presence of Doxicyclin, but in the absence of malignant cells (b); *in vitro*-differentiation in the presence of Doxicyclin and malignant cells Hela. A lot of cytoplasmic excrescences and cell-cell contacts are seen (c).

According to other literature findings, a calcium-dependent SCGN–TAU interaction, as well as co-appearance of both proteins is shown [12,21], despite the fact that two different genes code them. The absence of the mentioned above features in the process of myeloid differentiation in the presence of the received positive on additional copy of the oncogene *Dcn1* normal transfected cells could be accepted as a proof for the safety and immunogenity of these so derived transfected cells, which have preserved their non-tumorigenic/normal cell characteristics *in vitro*, as well as an indication about eventual over-expression of the experimentally-activated oncogene in genetically-manipulated normal cells. On the other hand, a rapid lymphoid-restricted (T-, B-, and NK) reconstitution capacity *in vivo*, as well as completely lacked myeloid differentiation potential both *in vivo* and/or *in vitro*, has been reported in stem cells from bone marrow material of adult laboratory mice [9,16].

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