Journal of Developmental Biology and Tissue Engineering Vol. 3(9), pp. 119-125, November 2011
Available online http://www.academicjournals.org/jdbte
ISSN 2141-2251 ©2011 Academic Journals
DOI: 10.5897/JDBTE11.017

Full Length Research Paper

Achievement of balanced oncogenes and tumor-suppressor genes activity in normal and malignant cells *in vitro* and *in vivo*

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

[1]Department of Experimental Morphology, Institute of Experimental Morphology, Pathology and Anthropology with Museum, Bulgarian Academy of Sciences, "Acad. G. Bonchev" Str., 1113 Sofia, Bulgaria.
[2]Institute of Biology and Immunology of Reproduction, Bulgarian Academy of Sciences, Sofia, Bulgaria

Accepted August 26, 2011

The main goal is connected with providing, on the one hand, of active tumor-suppressor genes for prevention of eventual malignant transformations, and, on the other hand, of functionally active oncogenes for prevention of early aging and death, both *in vitro* and *in vivo*. Modulation of an adequate immune control was also necessary, and in this way any eventual unwished side effects from the genetic manipulations applied, could be escaped. Gene transfer in laboratory-cultivated mouse embryonic stem cells (mESCs) was made by use of appropriate recombinant DNA-constructs, which contained the promoter for gene, coding Elongation Factor 1-alpha (EF1-α), isolated from adeno-associated virus (AAV) (Parvoviridae); gene Dcn1, isolated from 3T3 fibroblasts of laboratory mice Balb/c, as well as gene for neomycin resistance, isolated from bacterial DNA-plasmid. Besides those indicated in the scientific literature inactivation of oncogene Dcn1 in the process of normal cell differentiation, its presence in the genome was supported and confirmed by our results from electrophorhesis of genomic DNA from normal mature epithelial cells of adult Balb/c laboratory mice. Furthermore, electrophorhetic profiles of genetic material from wild type (WT) on oncogene Dcn1 and "knock-down" (KD) on it inbred lines experimental mice differed not only on this oncogene, but also on the tumor-suppressor gene HACE1 in both categories of laboratory rodents. Similarly transfected Hela and RIN-5F malignant cells were then *in vitro*-co-cultivated with myeloid cell precursors, derived from populations of non-transfected laboratory-cultivated mESCs, in the presence of Doxyciclin, known from many literature data as activator of tumor-suppressor genes from STAT-family expression. Our results were also confirmed by the noticed differences in the degree of myeloid differentiation of derived precursor cells in their *in vitro*-co-cultivation with containing additional copies of tumor-suppressor genes malignant cells from both lines described, in comparison with the data, obtained in their laboratory co-cultivation with non-treated human cervical carcinoma Hela cells. Differences were also observed in *in vitro*-co-cultivation with the derived by us normal mESCs, containing additional copy of oncogene Dcn1 by the described above transfection with recombinant DNA-constructs. On the other hand, the derived normal cells with inserted additional copy of oncogene Dcn1 have indicated good safety and immunogenity. These cells have also indicated preserved normal cell characteristics, as well as eventual over-expression of the experimentally-activated oncogene Dcn1 in them.

Key words: Oncogenes, tumor-suppressor genes, myeloid cell precursors, recombinant gene constructs, cell transfection.

INTRODUCTION

The importance of co-ordinated oncogenes and tumor-suppressor genes action in the regulation and prevention of malignant transformation has widely been investigated as important in the regulation and prevention of malignant transformations and of many age-related disorders (Wood et al., 2000; Zhang et al., 2007). On the other

*Corresponding author. E-mail: isainova@yahoo.com. Tel: + 359 2 8730210. Fax: + 359 2 8719007.

hand, according many literature findings genetic interactions between oncogene and tumor-suppressor genes (Bellosta et al., 2005; Etard et al., 2005), 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 (Bauer et al., 2000; Vogelstein and Kinzler, 2004), have been proven as possible. In this way, correlations of gene *p53* with gene *NUMB* has recently been indicated, characterized as a cell fate determinant because of its role in the asymmetric cell division in the mitosis process, as well as with gene *Oct4*, (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.

In the last years of XX century, gene therapy and tissue engineering have been found as the most important approaches, which exploit the current knowledge in molecular biology and biomaterial science in order to direct stem cells to *in vivo*-differentiation to desired lineages and tissues (Barrette et al., 2000; Borysiewicz et al., 1996; Brachmann et al., 1998; Chen et al., 2003; Domi and Moss, 2005). In this aspect, widely studied is the ability for *in vitro*-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 DNA- and RNA-viruses (Barrette et al., 2000; Borysiewicz et al., 1996), as well as bacterial plasmids and yeasts (Chen et al., 2003; Domi and Moss, 2005). For this aim, an intact gene *tk*, coding the enzyme timidinkinase (TK), has been found to be necessary, but, on the other hand – the integration of the searched gene(s) out of *tk* locus of the virus genome, as well as virus promoter, which could provide the expression of the inserted gene(s). Modifications by changes of the promoter and/or in the insertion site, as well as in the target vector repeats in fragments, expressing proteins with immunomodulator functions, have been proven to be possible. In this way have been inserted genes, coding cell receptors, cytokines, enzymes, complement activators, apoptosis activators and/or inhibitors, surface antigens, tumor markers. Besides the respective inserted gene(s), a marker gene has also been found to be necessary, but both gene types are controlled by appropriate promoter sequences. As a next step has been carried out polymerase chain reaction (PCR) of the received construction, by use of oligonucleotide primers for insurance of respective restriction sites – *SfiI*-site on the 5'-end and, respectively, *RsrII*-restriction site on the 3'-end of the PCR-product, which is obtained as a result of digestion by respective restriction enzymes (bacterial restrictases, which are particularly endonucleases), connected with respective early or late promoter in the virus genome or plasmid DNA.

Taking these data in consideration, the main goal was connected with providing of active tumor-suppressor genes for prevention of eventual malignant trans-formations, and, on the other hand, of active oncogenes for prevention of early aging and death, both *in vitro* and *in vivo*.

MATERIALS AND METHODS

Stem cells, isolated from mouse Balb/c embryos, were cultivated for 48 to 72 h 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*) (Chen et al., 2003), 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, were used. For gene transfer, 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 the indicated in the scientific literature activation of genes from *STAT*-family on its influence. At the same time, malignant cells from cell lines Hela and RIN-5F of human cervical carcinoma and rat insulinoma, containing additional copies of tumor-suppressor genes *HACE1* and Secretagogin gene, respectively, inserted by their transfection with recombinant gene constructs, were also cultivated and supported in analogical conditions. On the other hand, non-transfected cell cultures of the derived from human cervical carcinoma cell line Hela were also prepared. All cells were incubated at 37°C 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 they were 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 isolated nuclear genetic material was treated with lysis buffer (Sigma-Aldrich) for isolation of genomic DNA. The last was subjected on standart Polymerase Chain Reaction (PCR) of previously isolated nuclear DNA 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).

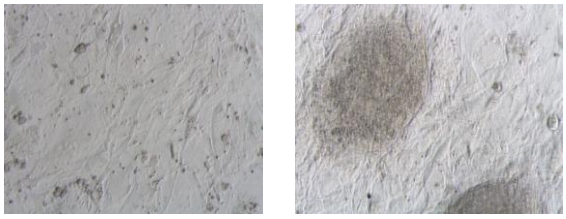
For differentiation in myeloid and lymphoid precursors, populations of non-transfected mESCs were further cultivated in medium, containing GM-CSF (Sigma-Aldrich) and complement proteins, respectively, by addition of 10% non-inactivated FCS (Sigma-Aldrich) in the last case.

Consequently, to the cell sub-populations of both non-transfected cell populations, malignant antigens were added. The last were derived by cultivation of Hela cells in serum-free DMEM (Sigma-Aldrich) for 24 h, its consequent centrifugation and filtration. Fixed light microscopic preparations were prepared by their consequent fixation 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 (Figures 1a and 1b).

According to the genomic assays results, 2 of the cell clones derived were positive on the additionally inserted



A B

Figure 1. Native light-microscopy preparations from transfected mESCs negative (a) and positive on additionally-inserted copy of the oncogene *Dcn1*, respectively (b).,

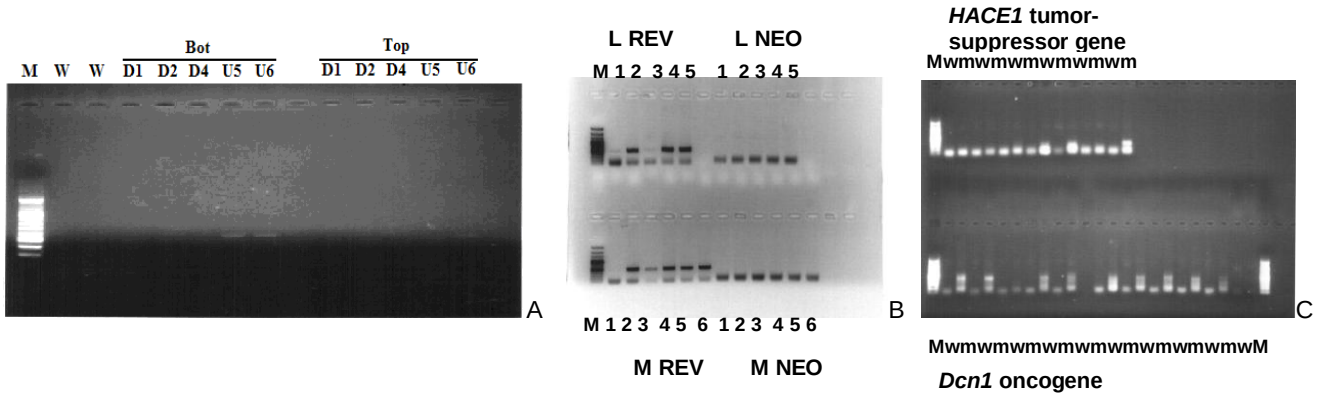


Figure 2. Agarose gel electrophorhesis for prove of the presence and/or the absence of additionally-inserted copy of the oncogene *Dcn1* in cell clones, derived from transfected by electroporation *in vitro*-cultivated mESCs (a) and in the used for cell transfection recombinant gene constructs (b); in mature epithelial cells, isolated from tail skin of adult experimental mice Balb/c with its normal expression (c), as well as from wild type (WT) and partially knock-down mutant (MT) on the same oncogene aboratory mice (d). Differences in the electrophorhetic profiles both of the oncogene *Dcn1* and the opposite tumor-suppressor gene *HACE1* could also be noted.

copy of the oncogene *Dcn1* and the other 7 cell clones - negative on it (Figure 2a). These results were confirmed by the data, obtained by electrophorhesis of the used recombinant DNA-constructs in the same conditions (Figure 2 – b). In genetic assay of nuclear DNA-material from high differentiated epithelial cells of homozygous on the oncogene *Dcn1* wild type (WT - *Dcn1*+/*Dcn1*+) and partially "knocked-down" on the same gene mutant heterozygous (MT - *Dcn1*+/*Dcn1*-) inbred lines of adult experimental mice, besides on it, differences in the electrophorhetic profiles of it, but also of the tumor-suppressor gene *HACE1*, were noticed (Figure 2 c).

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 2 μg/ml Doxicyclin (Sigma-Aldrich) (Figure 3).

These results could be confirmed by the observed signs of early myeloid and lymphoid differentiation in the presence of respective external factors (Figures 4 and 5).

According the results, the tendency for *in vitro*-differentiation in both myeloid and lymphoid precursors is stronger in the presence of transfected mESCs, containing additional copy of the oncogene *Dcn1*. Hence, the obtained data have also suggested that the so derived cells are safe enough both, and, on the other hand they have good immunogenic potential. The results obtained were compared with data, received from malignant rat insulinoma RIN-5F cells, containing additional copy of the Secretagogin gene, inserted by their transfection with recombinant gene construct *pGEX-1λT* (Amersham Pharmacia Biotech) of bacterial *Escherichia coli* strains, where a decreased malignant potential of the so transfected cells as a result of eventual induced Secretagogin over-expression, was supposed. As a proof about that could be accepted the observed effects of early myeloid differentiation and suppression on the cell proliferation in the presence of Doxicyclin could be explained with its activation effect on the tumor-suppressor genes of *STAT*-family. The data obtained

Figure 3. 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 (2 µg/ml - Sigma-Aldrich). Immune progenitor cells in different phases of immune differentiation, in particular, in different types myeloid precursors, most of which contain different types of granules in their cytoplasm, small cytoplasm amount with basophilic and/or eosinophilic granules, could be seen: *In vitro*-differentiation of non-transfected mESCs in the presence Doxicyclin, but in the absence of malignant cells (a); *In vitro*-differentiation in the presence of Doxicyclin and malignant cells Hela. A lot of cytoplasmic excrescences and cell-cell contacts are seen (b); *In vitro*-differentiation in the presence of GM-CSF and malignant cells Hela, containing tumor-suppressor gene *HACE1* (c); *In vitro*-differentiation in the presence of Doxicyclin and malignant cells RIN-5F, containing tumor-suppressor gene for Secretagogin (d); *In vitro*-differentiation in the presence of Doxicyclin and normal transfected mESCs, positive on additional copy of the oncogene *Dcn1* (e).

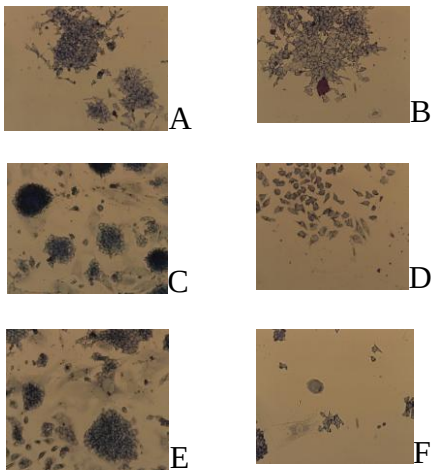


Figure 4. Early stages of myeloid *in vitro*-differentiation of non-transfected mESCs in different conditions: in the absence of differentiation factors and transfected cells (a); in the presence of GM-CSF, but in the absence of Hela-antigens and transfected cells (b); in the presence of Hela-antigens and in the absence of GM-CSF and transfected cells (c); in the presence of both GM-CSF and Hela-antigens, but in the absence of transfected cells (d); in the presence of GM-CSF, Hela-antigens and transfected cells, negative by additionally-inserted copy of oncogene *Dcn1* (e); in the presence of GM-CSF, Hela-antigens and transfected cells, positive on additionally-inserted copy of oncogene *Dcn1* (f).

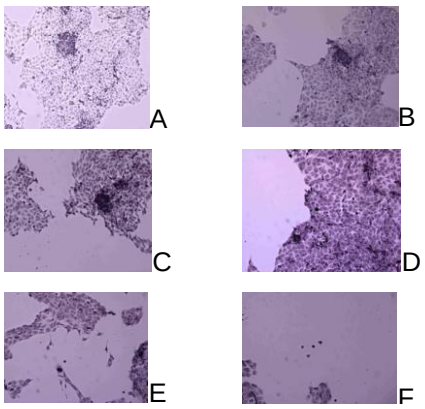


Figure 5. Early stages of lymphoid *in vitro*-differentiation of mESCs indifferent conditions: in the absence of differentiation factors and transfected cells (a); in the presence of complement components and absence of Hela-antigens and transfected cells (b); in the presence of Hela-antigens, but in the absence of complement proteins and transfected cells (c); in the presence of both complement components and Hela-antigens, but in the absence of transfected cells (d); in the presence of complement proteins, Hela-antigens and transfected cells, negative by additionally-inserted copy of oncogene *Dcn1* (e); in the presence of complement proteins, Hela-antigens and transfected cells, positive on additionally-inserted copy of oncogene *Dcn1* (f).

also slightly differed of the results, obtained in their laboratory co-cultivation with human cervical carcinoma non-transfected Hela cells in the same conditions, as well as in the absence of malignant cells, mainly in the number of formed cytoplasmic excrescences and contacts between cells by the so formed structures (Figure 4).

DISCUSSION

Despite of the fact that the expression of oncogene

*Dcn1*has been proof to be inhibited in the mature normal cells (Ma et al., 2008; O-charoenrat et al., 2008), the results obtained have supported its presence in their genomes (Figure 1, Figure 2 and Figure 3). These results were confirmed by previously published data, obtained from PCR and subsequent electrophoresis of the used recombinant vector constructs in the same conditions (Figure 2 - b), as well as of genomic DNA, isolated from mature epithelial cells from skin of adult Balb/c experimental mice (Figure 3 - a), and from normal wild type (WT) on the oncogene *Dcn1* and partially knock-down on it mutant (MT) adult laboratory rodents (Figure 3 – b). Taking in consideration literature data about the importance of coordinated oncogenes and tumor-suppressor genes action in the regulation and prevention of malignant transformation (Wood et al., 2000; Zhang et al., 2007), in both WT and knock-down MT on oncogene *Dcn1* in inbred experimental mice lines, respective electrophorhetic profiles of this gene, as well as of tumor-suppressor gene *HACE1* were made and compared, and the results obtained have indicated certain differences in both genes between the separated categories of laboratory rodents (Figure 3). The indicated 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 (Liang and Van Zant, 2003; Rubin, 1997; Vaziri and Benchimol, 1998; Vogelstein and Kinzler, 2004). 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* (Smith, 2001; Vaziri and Benchimol, 1998). The properties of “malignant stem cells”, have outlined initial therapeutic strategies against them (Smith and Boulanger, 2002; Vogelstein and Kinzler, 2004). A broad expression of oncogene *Dcn1*, characterized as a regulator of gene *p53*, has been detected in many tumor tissues and cultivated cell lines (Colaluca et al., 2008; Kurz et al., 2008; Ma et al., 2008; O-charoenrat et al., 2008). Function of this gene has also been found to be sufficient for cullin neddylation in a purified recombinant system, as well as, on the other hand – contribution of its over-expression to malignant disorders, as well as a potential marker for metastatic progression (Bowerman, 2007; Colaluca et al., 2008; Eferl et al., 2003; Gartner et al., 2007; Zhang et al., 2007). Links between DNA-replication, chromatin and proteolysis has been confirmed by the newly discovered cullin-RING E3-ubiquitin ligases, assembled on the CUL4 platform (Jin et al., 2006). In this aspect, a conserved component of CUL4-Dbd1 E3-ligase has been found as essential for the replication factor Cdt1 destruction and thus – for ensure proper cell cycle regulation of the DNA-replication process. Cullin-based E3-ligases, have recently been proven as crucial regulators of mitosis. A key role of the enzyme CUL7 E3-ubiquitin ligase in the proteolytic targeting insulin receptor substrate-1, which has been proven as a critical mediator for insulin/IGF1-signalling, has been demonstrated (Jin et al., 2006). On the other hand, both positive and negative roles of ubiquitin-mediated proteolysis in the regulation of longevity in the eukaryotic organism *Cenorhabditis elegans* by insulin/IGFs–signaling pathways, have been established (Bowerman, 2007). Studies on the biology of the stem cells are often focused on their self-renewal and differentiation (Amit et al., 2000; Coulombel, 2005; Cumano et al., 1992; Keller, 1995; Liang and Van Zant, 2003; Molofsky et al., 2004; Rubin, 1997; Smith, 2001; Vaziri and Benchimol, 1998; Vogelstein and Kinzler, 2004). 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 (Kobari et al., 2000). 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 4) (Fitzgerald et al., 2008; 2009; 2005; Kyba et al., 2003; Poehlmann et al., 2005; Suman et al., 2009). According to other literature findings, a calcium-dependent SCGN–TAU interaction, as well as co-appearance of both proteins is shown (Gartner et al., 2007; Maj et al., 2010; Wagner et al., 2000), despite the fact that two different genes code them. The noticed by us cytoplasmic excrescences and cell-cell-contacts in co-cultivation with non-transfected malignant Hela cells with no induced tumor-suppressor gene over-expression, known as signs of phagocyte cell differentiation, could be accepted as a proof for eventual decrease of the oncogene potential in malignant cells, containing additional copy of tumor-suppressor gene, in *in vitro*-conditions, as well as an indication about eventual over-expression of the experimentally-activated oncogene in genetically-manipulated normal cells. 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*.

Conclusion

The noticed cytoplasmic excrescences and cell-cell-contacts in co-cultivation with malignant cells are known as signs of phagocyte cell differentiation from the scientific literature. In this connection, the observed

highest degree of the formed structures in the presence of malignant Hela cells with no induced tumor-suppressor gene over-expression, could be accepted as a proof for eventual decrease of the oncogene potential in malignant cells, containing additional copy of tumor-suppressor gene, in *in vitro*-conditions. 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 transected cells could be accepted as a proof for the safety and immunogenity of these so derived cells, which have preserved their non-tumorigenic/normal cell characteristics *in vitro*, as well as for eventual over-expression of the experimentally-activated oncogene in genetically-manipulated normal cells.

REFERENCES

Amit M, Carpenter MK, Inokuma MS, Chiu CP, Harris CP, Waknitz MA, Itskovitz-Eldor J, Thomson JA (2000). Clonally derived human embryonic stem cell lines maintain pluripotency and proliferative potential for prolonged periods of culture. Dev. Biol., 227: 271-278.

Barrette S, Douglas JL, Seidel NE, Bodine DM (2000). *Lentivirus*-based vectors transduce mouse hematopoietic stem cells with similar efficiency to *moloney murine leukemia virus*-based vectors. Blood 96: 3385-3391.

Bauer A, Chauvet S, Huber O, Usseglio F, Rothbacher U, Aragnol D, Kemler R, Pradel J (2000). Pontin52 and reptin52 function as antagonistic regulators of beta-catenin signalling activity. EMBO J., 19: 6121-6130.

Bellosta P, Hulf T, Diop SB, Usseglio F, Pradel J, Aragnol D, Gallant P (2005). *Myc* interacts genetically with Tip48/Reptin and Tip49/Pontin to control growth and proliferation during *Drosophila* development. Proc. Nat. Acad. Sci. USA., 102: 11799-11804.

Borysiewicz LK, Flander A, Numako M, Man S, Wilkinson GW, Westmoreland D, Evans AS, Adams M, Stacey SN, Boursnell ME, Rutherford E, Hickling JK, Inglis SC (1996). A recombinant *vaccinia virus* encoding *human papillomavirus* types 16 and 18, E6 and E7 proteins as immunotherapy for cervical cancer. Lancet 347: 1523–1527.

Bowerman B (2007). *C. elegans* aging: proteolysis cuts both ways. Curr. Biol., 17: R514–R516.

Brachmann CB, Davies A, Cost GJ, Caputo E, Li J, Hieter P, Boeke JD (1998). Designer deletion strains derived from *Saccharomyces cerevisiae S288C*: a useful set of strains and plasmids for PCR-mediated gene disruption and other applications. Yeast 14: 115–132.

Chen S, Agarwal A, Glushakova OY, Jorgensen MS, Salgar SK, Poirier A, Flotte TR, Croker BP, Madsen KM, Atkinson MA, Hauswirth WW, Berns KI, Tisher CC (2003). Gene delivery in renal tubular epithelial cells using recombinant *adeno-associated viral* vectors. J. Am. Soc. Nephrol., 14: 947-958.

Colaluca IN, Tosoni D, Nuciforo P, Senic-Matuglia F, Galimberti V, Viale G, Pece S, Di Fiore PP (2008). NUMB controls *p53* tumor suppression activity. Nat. Lett., 451: 76–80.

Coulombel L (2005). Adult stem cells: who are they, what do they do? Bull. Acad. Natl. Med., 189: 589-602.

Cumano A, Paige CJ, Iskove NN, Brady G (1992). Bipotential precursors of B-cells and macrophages in murine fetal liver. Nat., 356: 612–615.

Domi A, Moss B (2005). Engineering of a *vaccinia virus* bacterial artificial chromosome in *Escherichia coli* by *bacteriophage λ*−based recombination. Nat. Meth., 2: 95–97.

Eferl R, Ricci R, Kenner L, Zenz R, David JP, Rath M, Wagner EF (2003). Liver tumor development. *c-Jun* antagonizes the proapoptotic activity of *p53*. Cell, 112: 181-192.

Etard C, Gradl D, Kunz M, Eilers M, Wedlich D (2005) Pontin andReptin regulate cell proliferation in early *Xenopus* embryos in collaboration with *c-Myc* and *Miz-1*. Mech. Dev., 122: 545-556.

Fitzgerald JS, Poehlmann TG, Schleussner E, Markert UR (2008) Trophoblast invasion: the role of intracellular cytokine signaling via signal transducer and activator of transcription 3 (STAT3). Trophoblast invasion: the role of intracellular cytokine signalling via signal transducer and activator of transcription 3 (STAT3) Human Reproduction Update, 14: 335–344.

Fitzgerald JS, Todd B, Jeschke U, Schleussner E, Markert UR (2009). Knocking off the suppressors of cytokine signaling (SOCS): their roles in mammalian pregnancy. J. Reprod. Immunol., 83: 117-123.

Fitzgerald JS, Tsareva SA, Poehlmann TG, Berod L, Meissner A, Corvinus FM, Wiederanders B, Pfitzner E, Markert UR, Friedrich K (2005). Leukemia inhibitory factor triggers activation of signal transducer and activator of transcription 3, proliferation, invasiveness, and altered protease expression in choriocarcinoma cells. Int. J. Biochem. Cell Biol., 37: 2284-2296.

Gartner W, Vila G, Daneva T, Nabokikh A, Koc-Saral F, Ilhan A, Majdic O, Luger A, Wagner L (2007). New functional aspects of the neuroendocrine marker secretagogin based on the characterization of its rat homolog. Am. J. Physiol. Endocrinol. Metab., 293: E347-E354.

Jin J, Arias EE, Chen J, Harper JW, Walter JC (2006). A family of diverse Cul4-Dbd1-interacting proteins includes Cdt2, which is required for S phase destruction of the replication factor Cdt1. Mol. Cell, 23: 709–721.

Keller GM (1995). *In vitro*-differentiation of embryonic stem cells. Curr. Opin. Cell. Biol., 7: 862-869.

Kobari L, Pflumio F, Giarratana M, Li X, Titeux M, Izac B, Leteurtre F, Coulombel L, Douay L (2000). *In vitro*- and *in vivo*-evidence for the long-term multilineage (myeloid, B-, NK, and T-) reconstitution capacity of *ex vivo*-expanded human CD34(+) cord blood cells. Exp. Hematol., 28: 1470-1480.

Kurz T, Chou YC, Willems AR, Meyer-Schaller N, Hecht ML, Tyers M, Peter M, Sicheri F (2008). Dcn1 functions as a scaffold-type E3 ligase for cullin neddylation. Mol. Cell, 29: 23–35.

Kyba M, Perlingeiro RCR, Hoover RR, Lu C-W, Pierce J, Daley GQ (2003). Enhanced hematopoietic differentiation of embryonic stem cells conditionally expressing Stat5. Proc. Nat. Aca. Sci. USA., 100: 11904-11910.

Liang Y, Van Zant G (2003). Genetic control of stem-cell properties and stem cells in aging. Curr. Opin. Hematol., 10: 195-202.

Ma T, Shi T, Huang J, Wu L, Hu F, He PF, Deng WW, Gao P, Zhang Y, Song Q, Ma D, Qiu X (2008). *DCUN1D3*, a novel UVC-responsive gene that is involved in cell cycle progression and cell growth. Mol. Cell, 99: 2128–2135.

Maj M, Gartner W, Ilhan A, Neziri D, Attems J, Wagner L (2010). Expression of TAU in insulin-secreting cells and its interaction with the calcium-binding protein secretagogin.J. Endocrinol., 205: 25-36.

Molofsky AV, Pardal R, Morrison SJ (2004). Diverse mechanisms regulate stem cell self-renewal. Curr. Opin. Cell. Biol., 16: 700-707.

O-charoenrat P, Sarkaria I, Talbot SG, Reddy P, Dao S, Ngai I, Shaha A, Kraus D, Shah J, Rusch V, Ramanathan Y, Singh B (2008). SCCRO (DCUN1D1) induces extracellular matrix invasion by activating matrix metalloproteinase 2. Clin. Cancer Res., 14: 6780-6789.

Poehlmann TG, Fitzgerald JS, Meissner A, Wengenmayer T, Schleussner E, Friedrich K, Markert UR (2005) Trophoblast invasion: tuning through LIF, signalling via *Stat3*. Placenta 26: S37-S41.

Rubin H (1997). Cell aging *in vivo* and *in vitro*. Mech. Ageing Dev., 98: 1-35.

Smith AG (2001). Embryo-derived stem cells: of mice and men. Annu. Rev. Cell. Dev. Biol., 17: 435-462.

Smith GH, Boulanger CA (2002). Mammary stem cell repertoire: new insights in aging epithelial populations. Mech. Ageing Dev., 123: 1505-1519.

Suman P, Poehlmann TG, Prakash GJ, Markert UR, Gupta SK (2009) Interleukin-11 increases invasiveness of JEG-3 choriocarcinoma cells by modulating *STAT3* expression. J. Reprod. Immunol., 82: 1-11.

Vaziri H, Benchimol S (1998). Reconstitution of telomerase activity in normal human cells leads to elongation of telomeres and extended replicative life span. Curr. Biol., 8: 279-282.

Vogelstein B, Kinzler KW (2004). Cancer genes and pathways they

control. Nat. Med. 10: 789–799.
Wagner L, Oliarnyc O, Gatner W, Nowotny P, Groeger M, Kaserer K, Waldhausl W, Paternack MS (2000). Cloning and expression of secretagogin, a novel neuroendocrine- and pancreatic islets of Langerhans– specific Ca^{2+} binding protein. J. Biol. Chem., 275: 24740–24751.
Wood MA, McMahon SB and Cole MD (2000). An ATPase/helicase complex is an essential cofactor for oncogenic transformation by c-Myc. Mol. Cell, 5: 321-330.
Zhang L, Anglesio MS, O'sullivan M, Zhang F, Yang G, Sarao R, Ngheim MP, Cronin S, Hara H, Melnyk N, Li L, Wada T, Liu PP, Farrar J, Arceci RJ, Sorensen PH, Penninger JM (2007). The E3 ligase HACE1 is a critical chromosome *6q21* tumor suppressor involved in multiple cancers. Nat. Med., 13: 1060-1069.