doi:10.4149/neo_2009_06_542

Genotoxic damage of human adipose-tissue derived mesenchymal stem cells triggers their terminal differentiation

V. ALTANEROVA[1], E. HORVATHOVA[2], M. MATUSKOVA[1], L. KUCEROVA[1], C. ALTANER[1]*

[1]*Laboratory of Molecular Oncology,* [2]*Laboratory of Mutagenesis and Carcinogenesis, Cancer Research Institute SAS, Bratislava, Slovakia, e-mail: exonalt@savba.sk*

Received April 26, 2009

Human adipose tissue-derived mesenchymal (stromal) stem cells (AT-MSCs) and genetically modified to express cytosine deaminase:uracil phosphoribosyltransferase (CDy-AT-MSCs) were treated with hydrogen peroxide in order to induce DNA damage and subsequently evaluate their genetic stability by single cell gel electrophoresis. Both cells types (parental and transgene modified) did not differ in the sensitivity to DNA breaks induction. Potential tumorigenicity of AT-MSCs and CDy-AT-MSCs was tested by subcutaneous inoculation of cell suspension into flank of immunocompromised mice. Dose of $15x10^6$ cells was not found to be tumorigenic in given experimental setup. AT-MSCs, CDy-AT-MSCs and MSCs isolated from human lipoma were treated with chemical carcinogen 4-nitroquinoline-1-oxide (4NQO) in attempts to transform them. Surviving cells after genotoxic stress were not transformed but underwent replicative senescence. Irreparable DNA damage caused triggered adipogenic terminal differentiation, rather than apoptosis induction in all kinds of cells tested.

Key words: human adipose tissue-derived mesenchymal stem cells; genotoxic damage, single cell gel electrohoresis; chemical carcinogen 4NQO, terminal differentiation

Human adipose tissue-derived mesenchymal (stromal) stem cells (AT-MSCs) are multipotent adult stem cells derived usually from lipoaspirate. Similarly as bone marrow derived mesenchymal stem cells (MSC), AT-MSCs have the ability to migrate to sites of injury to fulfill their role in repair of the damage tissues [1]. MSCs and AT-MSCs can be isolated by their adherence to plastic tissue culture plates [2] The yield of MSCs from adipose tissue is higher in comparison to the bone marrow [1, 3, 4]. The easy and repeatable access to subcutaneous adipose tissue and the simple isolation procedures provides a clear advantage over other sources [4]. Therefore AT-MSCs have potential therapeutic use as autologous and allogeneic products for tissue engineering.

Mesenchymal stem cells both MSCs and AT-MSCs possess unique ability to selectively migrate to tumors, metastases and contribute to the formation of tumor-associated stroma. This property predetermines them to become vehicles for stem cell based targeted cancer gene therapy [5, 6].

Previously we have prepared yeast fusion cytosine deaminase:uracil phosphoribosyltransferase expressing human adipose tissue derived mesenchymal stem cells (CDy-AT-MSCs) by retrovirus transduction. CDy-AT-MSCs exerted their anti-tumor potential on human colon cancer cells, human malignant melanoma cell line, and human metastatic prostate cells in the presence of prodrug 5-fluorocytosine (5-FC) [3, 7, 8]. CDy-AT-MSCs in combination with 5-FC augmented significant bystander effect and selective cytotoxicity on target tumor cells in co-culture *in vitro*. Moreover, strong inhibition of subcutaneous tumor growth was achieved by systemically administered CDy-AT-MSCs on xenotransplanted human tumors grown on nude mice upon 5-FC treatment.

Therefore human mesenchymal stem cells derived from adipose tissue are not only useful cells in regenerative medicine, but when modified by prodrug converting gene may also serve as attractive delivery vehicles for stem cell-based targeted cancer gene therapy. Genetic stability of AT-MSCs is an important attribute to asses, when they are intended to be used in regenerative and cancer gene therapy clinical studies.

The aim of the present study was to evaluate genetic stability, of AT-MSCs and CDy-AT-MSCs, their sensitivity to transformation induced by chemical carcinogen and potential tumorigenicity. Here we demonstrate that irreparable DNA damage caused by chemical carcinogen triggered adipose

* **Corresponding author**

derived mesenchymal (stromal) cells to adipogenic, terminal differentiation.

Materials and methods

Chemicals. Ethidium bromide, 4-nitroquinoline-1-oxide (4NQO) were purchased from Sigma-Aldrich Co. (St. Louis, MO). 4NQO was prepared as 5 mmol/L stock solutions in 100% dimethylsulfoxide (DMSO). Aliquots were diluted with complete medium just before they were applied to culture cells.

Mesenchymal stem cells isolation from human adipose tissue, culture, and retrovirus transduction. AT-MSC cells were isolated and cultivated as we described previously [7]. Lipoaspirates for their isolation were obtained from healthy persons undergoing elective lipoaspiration, who provided an informed consent. Cells were plated in low glucose (1,0 mg/L) DMEM supplemented with 5% MSC-stimulatory supplement (StemCell Technologies), 5% of human platelet extract and antibiotic-antimycotic mixture. AT-MSCs were cultured at 37^0C in a humidified atmosphere containing 5% carbon dioxide with medium changes twice a week. Cells were plated at a density of $3x10^3$ to $5x10^3$ nucleated cells/cm^2. Adherent cells were split upon reaching confluence and AT-MSCs were used for the experiments up to passage 5.

To prepare AT-MSCs expressing cytosine deaminase (CDy-AT-MSCs), subconfluent cultures of AT-MSCs were transduced thrice in 3 consecutive days with virus-containing medium from GP+envAM12/pST2 cells supplemented with 100 μg/mL protamine sulphate. Transduced cells were selected by cultivation in medium supplemented with 0.5 mg/ml of G418.

Experiments in vivo. Six- to 8-week-old athymic nude mice (Balb/c-nu/nu) were used in accordance with institutional guidelines under the approved protocols. The potential tumorigenicity of AT-MSCs and CDy-AT-MSCs was tested by subcutaneous inoculation of cell suspension of $15x10^6$ AT-MSCs or CDy-AT-MSCs in 0.1 ml PBS into flank of each nude mouse (n = 2 in each treatment group). The animals were inspected for tumor growth during 60 days and then the tumor presence was control by autopsy.

Hydrogen peroxide treatment and DNA-damage testing. The method of single cell gel electrophoresis (SCGE; comet assay) according to [9] was performed with minor modifications suggested in [10, 11]. Briefly: 2.5 x 10^4 AT-MSCs or CDy-AT-MSCs in 85μl of 0.75% low melting point agarose in PBS were spread on a base layer of 100 μl 1% normal-melting point agarose in PBS, placed on microscope slides and covered with a cover slip. When the gel solidified, the cover slip was removed. The cells were then exposed to different concentrations of hydrogen peroxide (0, 100, 200, 300, and 400 μM). Hydrogen peroxide treatment (5 min on ice) minimizes the DNA repair process [12]. The slides were washed with PBS and placed in lysis solution (2.5 M NaCl, 0.1 M Na_2EDTA, 10 mM Tris–HCl, pH 10.0, 1% Triton X-100) for 1 h at 4 ^{0}C. After lysis the slides were transferred to an electrophoresis buffer (0.3 M NaOH, 1 mM Na_2EDTA, pH > 13.0) for unwinding (40 min at 4^0C) and then subjected to electrophoresis at 25 V (current adjusted to 0.3 A) for 30 min at 4^0C. The slides were neutralized with 0.4 M Tris–HCl (pH 7.5) and stained with ethidium bromide (EtBr, 5μg/ml). For each sample, 100 EtBr-stained nucleoids on triplicate slides were evaluated and scored with an Olympus fluorescent microscope and computerized image analysis (Komet 5.5, Kinetic Imaging, Liverpool, UK) for determination of DNA in the tail, which is linearly related to the frequency of single strand DNA breaks

Treatment of AT-MSCs, CDy-AT-MSCs, and MSCs from lipoma with 4NQO.

Cells were exposed to single dose of 10 μM concentration of 4NQO. Survived fraction of cells (about ten percent) was further cultivated in growth medium and when reached confluence transferred by trypsin treatment. The cultivation continued for several months. During this period of time signs of senescence appeared, intervals of cell transfer was longer, the number of passages never reach more than 10 before the terminal differentiation appeared.

Statistics. Statistical comparisons of mean tail DNA values in hydrogen peroxide treatment experiments were performed using the Student's t-test.

Results

Induction of single stranded DNA breaks. Treatment of AT-MSCs and CDy-AT-MSCs with hydrogen peroxide resulted in an increase of DNA breaks in both parental AT-MSCs and transgene-modified CDy-AT-MSCs when concentration of hydrogen peroxide reached 100 μM. Higher hydrogen peroxide concentration did not further increase the number of DNA breaks (Fig. 1.) Percentage of tail DNA was comparable at each hydrogen peroxide concentration tested in both kinds of cells. Therefore the genetically modified mesenchymal cells did not differ from parental cells in the sensitivity to DNA breaks induction.

Tumorigenicity of AT-MSCs and CDy-AT-MSCs testing. Inoculums of cell suspension ($15x10^6$ of AT-MSCs or CDy-AT-MSCs) in 0.1 ml PBS was injected subcutaneously into the flank of nude mouse (n = 2 in each treatment group). Animals were inspected for tumor growth during period of time two months. The palpable cell inoculum persisted on the site of injection several days and slowly disappeared during first two weeks. By day 60 the experiments were ended and animals were sacrificed and ispected for tumor presence. No tumors were found either on the site of inoculation nor elsewhere during the autopsy. Therefore the AT-MSCs and CDy-AT-MSCs were found not to be tumorigenic in given experimental setup.

Cytotoxicity of 4NQO to AT-MSC and CDy-AT-MSC. Mesenchymal stem cells are known for their higher natural resistance to toxic agents. In order to elucidate the sensitivity

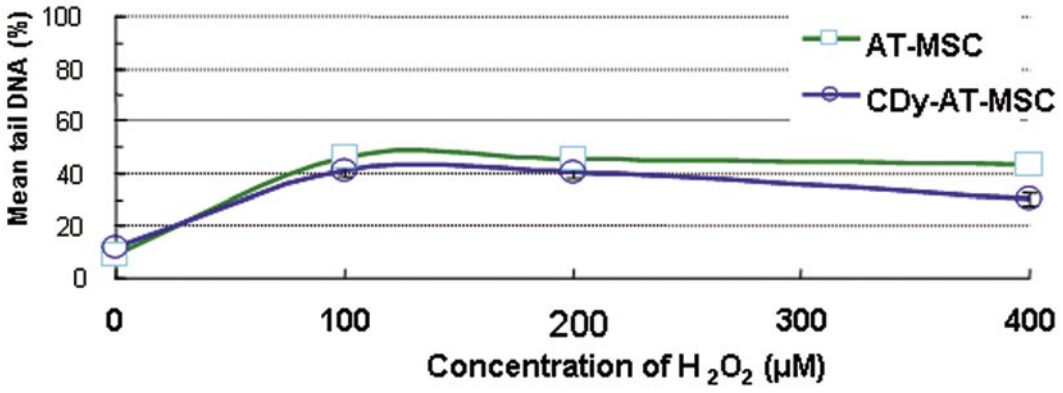

Figure 1. DNA-damaging effect of hydrogen peroxide on parental and cytosine deaminase transduced human adipose tissue derived mesenchymal stem cells. Details of experimental procedure and evaluation is described in Material and Methods.

of AT-MSCs, CDy-AT-MSCs and MSCs isolated from lipoma, potent chemical carcinogen and mutagen 4-nitroquinoline-1-oxide (4NQO) was chosen for cell treatment. Concentrations higher than 60 μM of 4NQO were found to be toxic to destroy cells completely during 4 days of cultivation. At least 10 percent of cells survived treatment with 10 μM concentration of 4NQO for 4 days. Recovered 4NQO-treated cells were further cultivated and split upon reaching confluence. Cell proliferation declined with passages kept in culture. Cells became more light-refractory, the cell morphology changed to cells more prolonged (Fig. 2 B). Typical signs of aging-related phenotype appeared (Fig. 2 C). Finally, the cells appeared to form drops of fat, signs of mature differentiation to adipocytes in all cells (Fig. 2 D). Accumulation of irreparable DNA damage in adipose tissue derived mesenchymal stem cells apparently triggered their terminal differentiation. Terminal adipogenic differentiation after genotoxic stress caused by 4NGO was noticed in AT-MSCs, in cytosine deaminase transduced CDy-ATMSCs and also in MSCs isolated from human lipomas.

Discussion

Several properties of human mesenchymal stem cells derived from adipose tissue designate them attractive vehicles for cell therapies. They are able to differentiate along a variety of lineage pathways, including bone, cartilage, adipose, neuronal-like, and muscle *in vitro* [see 1 for a review]. They are nonimmunogenic upon transplantation into allogeneic host [4, 13, 14]. The cells exhibit a low immunogenic profile as shown by negative expression of MHC class II molecules and absence of costimulatory molecule expression [15, 16, 17]. Furthermore it was found that they are immunosuppressive [18, 19] as it was demonstrated by their ability to control graft-versus-host disease (GvHD) in humans [20].

Moreover AT-MSCs possess unique ability to selectively migrate to tumors and metastases. When AT-MSCs are modified by prodrug converting gene they are attractive delivery vehicles for anti-tumor agents such as IFNβ to tumors [21] and for stem cell-based targeted cancer gene therapy [3, 7, 8, 22]. Taken in account nonimmunogenic properties of MSCs, AT-MSCs may have potential therapeutic use as allogeneic products for tissue engineering and for gene therapy upon genetic modifications as well. For all these reasons, to assess genetic stability of these cells is rather important, when they are intended to be used in clinical studies.

There were several contradictive reports in literature with regards to involvement of adult MSCs in tumor formation [23]. Increasing number of reports indicated that MSCs are recruited in large numbers to the stroma of developing tumors [21, 24], or they could act by enhancing the motility, invasion and metastasis ability of adjacent cancer cells [25].

Experiments with human xenografts on nude mice have shown that systemically administrated MSCs possess intrinsic preferential migratory ability towards breast carcinoma cells, lung metastasis of melanoma cells, intracranial glioma and colon cancer cells [26, 27]. Moreover exogenously administered MSCs could form a significant proportion of tumor mass [27]. It was demonstrated that bone marrow derived MSCs may also be involved in cancer metastasis forming pre-metastatic niche [28]. This finding supports the idea that therapeutically engineered MSCs may be used to treat metastases. Therefore the introduction of a transgene into autologous stem cells possesses an attractive cell based delivery strategy [8, 29]. On the contrary, other studies observed that MSCs may inhibit tumor growth in animal models [30, 31] and posses antitumorigenic effects on a model of Kaposi's sarcoma [32].

Few contradictory reports appeared about spontaneous transformation of cultured bone marrow derived murine MSCs [32, 33, 34, 35] and human MSCs [36, 37]. It was

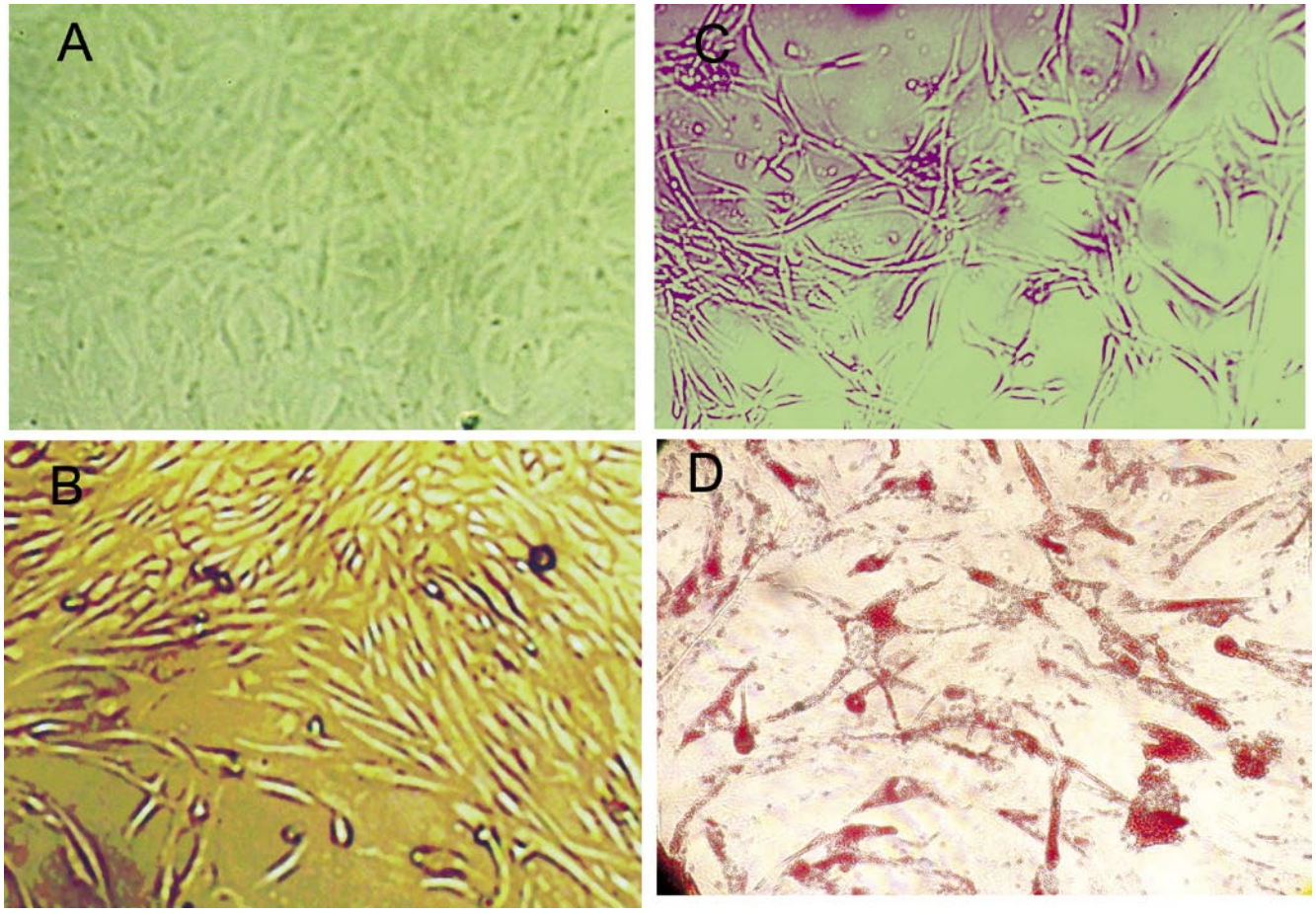

Figure 2. Adipogenic terminal differentiation of AT-MSC after accumulation of DNA damage caused by 4NQO. A – untreated control AT-MSCs; B – AT-MSCs exposed to 4NQO after serial passages became bigger; C- Senescence of damaged AT-MSCs progressed during serial passages, cells being more light refractory and sparse; D – All 4NQO-treated AT-MSCs differentiated to adipocytes. Cells were stained with Oil Red-O. Magnification 100 fold.

reported that murine but not human mesenchymal stem cells generated osteosarcoma-like lesions in the lung [38]. If murine MSCs were implanted subcutaneously together with porous ceramic, host-derived sarcomas developed. Sarcomas developed only in syngenic and immunodeficient recipients, but not in allogenic hosts or when the cells were inoculated as suspension [39].

In our experiments we did not observed any tumor formation when a high dose of AT-MSCs, or CDy-AT-MSCs was inoculated subcutaneously as cell suspension in nude mice. However, when these cells are co-injected with tumor cells, they can support the growth of engrafts of human melanoma xenografts, but not human glioma cells on nude mice, due to their production of various growth factors in a paracrine fashion [40].

Mesenchymal stem cells as cells of vital importance for organism are equipped with efficient DNA repair system as well as high natural resistance to toxic agents [41]. It was therefore not surprising that DNA damage in CDy-AT-MSCs was not influenced by the transduction with cytosine deaminase as documented by our results obtained with single-cell gel electrophoresis, which represents a sensitive method for measuring DNA damage.

In order to find whether adipose tissue derived mesenchymal stem cells can be transformed *in vitro* by chemical carcinogen, we have chosen water soluble highly effective carcinogen 4-nitroquinoline-1-oxide (4NQO). 4NQO is metabolized into 4-acetoxyaminoquinoline-1-oxide (Ac-4HAQO), which can form covalent adduct to deoxyguanine and deoxyadenine in DNA. The carcinogenic activity of 4NQO consist in DNA damage production and formation of irreversible topoisomerase I cleavage complexes (Top1cc) inducing recombinations. Top1cc produced by 4NQO accumulate progressively after 4NQO addition and persist to following 4NQO removal. [42]. In addition 4NQO also produces oxidative damage and DNA single-stranded breaks [43]. Results of our study indicate that replicative senescence of AT-MSCs as a consequence of cumulating DNA damage is a continuous process including far reaching alterations in phenotype like the terminal differentiation. Similar observation with human

bone marrow derived MSC together with detail analysis on molecular level was reported [44, 45]. The DNA damage, a prime suspect in stem cell aging, causes graying and loss of melanocyte stem cells by inducing premature differentiation, without inducing apoptosis or senescence as it was reported very recently [46]. The irreparable DNA damage, as caused by ionizing radiation, abrogated renewal of MSCs in mice. Surprisingly, the DNA-damage response triggers MSC differentiation into mature melanocytes in the niche, rather than inducing their apoptosis or senescence [46].

Several other reports support the conclusion that accumulation of DNA damage may cause somatic stem cell depletion [47, 48]. Typical sign of aging in mammals is hair graying caused by the incomplete maintenance of melanocyte stem cells with age [47]. The resulting MSC depletion leads to irreversible hair graying. Deficiency of Ataxia-telangiectasia mutated (ATM), a central transducer kinase of the DNA-damage response, sensitizes MSCs to ectopic differentiation, demonstrating that the kinase protects MSCs from their premature differentiation by functioning as a "stemness checkpoint" to maintain the stem cell quality and quantity [49].

We observed that genotoxic damage of human adipose tissue derived mesenchymal stem cells caused by chemical carcinogen 4NQO is not leading to cell transformation but to process of ageing. The aged AT-MSCs are triggered to adipogenic terminal differentiation. These data are in agreement with above mentioned observations of terminal differentiation of melanocyte stem cells [46]. Whether the elimination of DNA damaged stem cells is generally provided by terminal differentiation rather than apoptosis remains to be proven.

Acknowledgements. The study was supported by APVV grant 0260/07 (awarded to L. K.); VEGA grant 2/0072/09 (awarded to E. H.); and financial supports awarded to C. A. from SPP Foundation, FIDURA Capital Consult GmbH, Munich, Germany; and the League Against Cancer. We thank D. Guba M.D., Institute of Medical Cosmetics, Bratislava, Slovakia for providing us with material for AT-MSC isolation, M. Dubrovcakova and V. Frivalska for technical assistance.

References

[1] SCHAFFLER A, BUCHLER C. Concise review: adipose tissue-derived stromal cells – basic and clinical implications for novel cell-based therapies. Stem Cells 2007; 25: 818–827. doi:10.1634/stemcells.2006-0589

[2] ZUK PA, ZHU M, ASHJIAN P et al. Human adipose tissue is a source of multipotent stem cells. Mol Biol Cell 2002; 13: 4279–4295. doi:10.1091/mbc.E02-02-0105

[3] KUCEROVA L, ALTANEROVA V, MATUSKOVA M et al. Adipose tissue derived human mesenchymal stem cells mediated prodrug cancer gene therapy. Cancer Res 2007; 67: 6304–6313. doi:10.1158/0008-5472.CAN-06-4024

[4] KERN S, EICHLER H, STOEVE J et al. Comparative analysis of mesenchymal stem cells from bone marrow, umbilical cord blood or adipose tissue. Stem Cells 2006; 24: 1294–1301. doi:10.1634/stemcells.2005-0342

[5] ALTANER C. Prodrug cancer gene therapy. Cancer Letters 2008; 270: 191–201. doi:10.1016/j.canlet.2008.04.023

[6] ALTANER C. Glioblastoma and stem cells Minireview. NEOPLASMA 2008; 55: 369–374.

[7] KUCEROVA L, MATUSKOVA M, PASTORAKOVA A et al. Cytosine deaminase expressing human mesenchymal stem cells mediated tumour regression in melanoma bearing mice. J Gene Med 2008; 10: 1071–1082. doi:10.1002/jgm.1239

[8] CAVARRETTA I, ALTANEROVA V, MATUSKOVA M et al. Adipose tissue-derived mesenchymal stem cells expressing prodrug converting enzyme inhibit human prostate tumor growth. Molecular Therapy (2009, in revision)

[9] SINGH, NP, MCCOY MT, TICE RR et al. A simple technique for quantitation of low levels of DNA damage in individual cells. Exp. Cell Res.1988; 175: 184–191. doi:10.1016/0014-4827(88)90265-0

[10] SLAMENOVA D, GABELOVA A, RUZEKOVA L et al. Detection of MNNG-induced DNA lesions inmammalian cells: validation of comet assay against DNA unwinding technique,alkaline elution of DNA and chromosomal aberrations. Mutat. Res. 1997; 383: 243–252.

[11] SLAMENOVA D, HORVATHOVA E, MARSALKOVA L, et al. Carvacrol given to rats in drinking water reduces the level of DNA lesions induced in freshly isolated hepatocytes and testicular cells by H2O2. Neoplasma. 2008; 55: 394–399.

[12] GABELOVA A, SLAMENOVA D, RUZEKOVA L et al. Measurement of DNA strand breakage and DNA repair induced with hydrogen peroxide using single cell gel electrophoresis, alkaline DNA unwinding and alkaline elution of DNA. Neoplasma 1997; 44: 380–388.

[13] STREM BM, HICOK KC, ZHU M et al. Multipotential differentiation of adipose tissue-derived stem cells. Keio J.Med. 2005; 54:132–141. doi:10.2302/kjm.54.132

[14] KEATING A. Mesenchymal stromal cells. Curr. Opin. Hematol. 2006; 13: 419–425. doi:10.1097/01.moh.0000245697.54887.6f

[15] MCINTOSH K, ZVONIC S, GARRETT S et al. The immunogenicity of human adipose derived cells: Temporal changes in vitro. Stem Cells 2006; 24: 1246–1253. doi:10.1634/stemcells.2005-0235

[16] NIEMEYER P, KORNACKER M, MEHLORN A et al. Comparison of immunological properties of bone marrow stromal cells and adipose tissue derived stem cells before and after osteogenic differentiation in vitro. Tissue Eng 2007; 13: 111–121. doi:10.1089/ten.2006.0114

[17] CROP MJ, BAAN CC, KOREVAAR SS. et al. Donor-derived mesenchymal stem cells suppress alloreactivity of kidney transplant patients. Transplantation. 2009; 87: 896–906. doi:10.1097/TP.0b013e31819b3d72

[18] PUISSANT B., BARREAU C., BOURIN P et al. Immunomodulatory effect of human adipose tissue derived adult stem cells: comparison with bone marrow mesenchymal stem cells. Br J Haematol 2005; 129: 118–129. doi:10.1111/j.1365-2141.2005.05409.x

[19] CUI L, YIN S, LIU W et al. Expanded adipose derived stem cells suppress mixed lymphocyte reaction by secretion of prostaglandin E2. Tissue Eng 2007; 13: 1185–1195. doi:10.1089/ten.2006.0315

[20] YAÑEZ R, LAMANA ML, GARCÍA-CASTRO J et al. Adipose tissue-derived mesenchymal stem cells have in vivo immunosuppressive properties applicable for the control of the graft-versus host disease. Stem Cells 2006; 24: 2582–2591. doi:10.1634/stemcells.2006-0228

[21] STUDENY FC, MARINI JL, DEMBINSKI C et al. Mesenchymal stem cells: potential precursors for tumor stroma and targeted-delivery vehicles for anticancer agents, J. Natl. Cancer Inst. 2004; 96: 1593–1603.

[22] ABOODY KS, BUSH RA, GARCIA E, et al. Development of a tumor selective approach to treat metastatic cancer. PLoS ONE 2006;1: E23–31. doi:10.1371/journal.pone.0000023

[23] LAZENNEC G, JORGENSEN C Concise review: adult multipotent stromal cells and cancer: risk or benefit? Stem Cells. 2008; 26: 1387–1394. doi:10.1634/stemcells.2007-1006

[24] HUNG SC, DENG WP, YANG WK et al. Mesenchymal stem cell targeting of microscopic tumors and tumor stroma development monitored by noninvasive in vivo positron emission tomography imaging. Clin. Cancer Res.2005; 11: 7749–7756. doi:10.1158/1078-0432.CCR-05-0876

[25] KARNOUB AE, DASH AB, VO AP et al. Mesenchymal stem cells within tumour stroma promote breast cancer metastasis. Nature, 2007; 449: 557–563. doi:10.1038/nature06188

[26] STUDENY M, MARINI FC, CHAMPLIN RE et al. Bone marrow-derived mesenchymal stem cells as vehicles for interferon-beta delivery into tumors, Cancer Res. 2002; 62: 3603–3608.

[27] NAKAMIZO A. MARINI F, AMANO T et al. Human bone marrow-derived mesenchymal stem cells in the treatment of gliomas. Cancer Res. 2005; 65: 3307–3318.

[28] KAPLAN RN, PSAILA B, LYDEN D Bone marrow cells in the'pre-metastatic niche': within bone and beyond, Cancer Metastasis Rev 2006; 25: 521–529. doi:10.1007/s10555-006-9036-9

[29] HAMADA H, KOBUNE M, NAKAMURA K et al. Mesenchymal stem cells (MSC) as therapeutic cytoreagents for gene therapy, Cancer Sci. 2005; 96: 149–156. doi:10.1111/j.1349-7006.2005.00032.x

[30] RAMASAMY R, LAM EW, SOEIRO I. et al. Mesenchymal stem cells inhibit proliferation and apoptosis of tumor cells: impact on in vivo tumor growth. Leukemia 2007; 21: 304–310. doi:10.1038/sj.leu.2404489

[31] OHLSSON LB, VARAS L, KJELLMAN C, et al. Mesenchymal progenitor cell-mediated inhibition of tumor growth in vivo and in vitro in gelatin matrix. Exp Mol Pathol. 2003; 75: 248–255. doi:10.1016/j.yexmp.2003.06.001

[32] KHAKOO AY, PATI S, ANDERSON SA et al. Human mesenchymal stem cells exert potent antitumorigenic effects in a model of Kaposi's sarcoma, J. Exp. Med. 2006; 203: 1235–1247. doi:10.1084/jem.20051921

[33] MIURA M, MIURA Y, PADILLA-NASH HM, et al. Accumulated chromosomal instability in murine bone marrow mesenchymal stem cells leads to malignant transformation. Stem Cells. 2006; 24: 1095–1103. doi:10.1634/stemcells.2005-0403

[34] TOLAR J, NAUTA AJ, OSBORN MJ, et al. Sarcoma derived from cultured mesenchymal stem cells. Stem Cells. 2007; 25: 371–379. doi:10.1634/stemcells.2005-0620

[35] ZHOU YF, BOSCH-MARCE M, OKUYAMA H, et al. Spontaneous transformation of cultured mouse bone marrow-derived stromal cells. Cancer Res. 2006; 66: 10849–10854. doi:10.1158/0008-5472.CAN-06-2146

[36] RUBIO D, GARCIA-CASTRO J, MARTÍN MC, et al. Spontaneous human adult stem cell transformation. Cancer Res. 2005; 65: 3035–3039.

[37] RØSLAND GV, SVENDSEN A, TORSVIK A, et al. Long-term cultures of bone marrow-derived human mesenchymal stem cells frequently undergo spontaneous malignant transformation. Cancer Res. 2009; 69: 5331–5339. doi:10.1158/0008-5472.CAN-08-4630

[38] AGUILAR S, NYE E, CHAN J, et al. Murine but not human mesenchymal stem cells generate osteosarcoma-like lesions in the lung. Stem Cells. 2007; 25: 1586–1594. doi:10.1634/stemcells.2006-0762

[39] TASSO R, AUGELLO A, CARIDA M et al. Development of sarcomas in mice implanted with mesenchymal stem cells seeded onto bioscaffolds. Carcinogenesis 2009; 30: 150–157. doi:10.1093/carcin/bgn234

[40] KUCEROVA L, MATUSKOVA, M. HLUBINOVA, K et al. Human adipose tissue-derived mesenchymal stromal cells as modulators of tumor microenvironment. Molecular Cancer (2009, submitted).

[41] HILDRESTRAND GA, DUGGAL S, BJØRÅS M, et al. Modulation of DNA glycosylase activities in mesenchymal stem cells. Exp Cell Res. 2009 May 25. [Epub ahead of print]

[42] MIAO ZH, RAO VA, AGAMA K et al. 4-Nitroquinoline-1-Oxide induces the formation of cellular topoisomerase I-DNA cleavage complexes. Cancer Res. 2006; 66: 6540–6545. doi:10.1158/0008-5472.CAN-05-4471

[43] NAGAO M, SUGIMURA T. Molecular biology of the carcinogen, 4-nitroquinoline 1-oxide. Adv Cancer Res 1976; 23: 131–169. doi:10.1016/S0065-230X(08)60545-X

[44] WAGNER W, HORN P, CASTOLDI M, et al. Replicative senescence of mesenchymal stem cells: a continuous and organized process. PLoS One. 2008; 3: e2213. doi:10.1371/journal.pone.0002213

[45] WAGNER W, BORK S, HORN P, et al. Aging and replicative senescence have related effects on human stem and progenitor cells. PLoS One. 2009; 4: e5846. doi:10.1371/journal.pone.0005846

[46] INOMATA K, AOTO T, BINH NT et al. Genotoxic stress abrogates renewal of melanocyte stem cells by triggering their differentiation. Cell. 2009; 137: 1088–1099. doi:10.1016/j.cell.2009.03.037

[47] RUZANKINA Y, PINZON-GUZMAN C, Asare A, et al. Deletion of the developmentally essential gene ATR in adult mice leads to age-related phenotypes and stem cell loss. Cell Stem Cell. 2007; 1: 113–126. doi:10.1016/j.stem.2007.03.002

[48] INOUE-NARITA T, HAMADA K, SASAKI T, et al. Pten deficiency in melanocytes results in resistance to hair graying and susceptibility to carcinogen-induced melanomagenesis. Cancer Res. 2008; 68: 5760–5768. doi:10.1158/0008-5472.CAN-08-0889

[49] NISHIMURA EK, GRANTER SR, FISHER DE Mechanisms of hair graying: incomplete melanocyte stem cell maintenance in the niche. Science. 2005; 307: 720–724. doi:10.1126/science.1099593