Pap Test as the First Step in Screening Genetic Stability in Cell-Based Therapy

Ana C. Irioda1, Larissa Zocche2, Carolina M. C. O. Souza2, Reginaldo J. Ferreira4, Eduardo Aliprandini2, Ricardo C. Cunha3, Julio C. Francisco5, Luiz C. Guarita-Souza3, Mariester Malvezzi5, Miriam P. Beltrame6, Lismay A. F. Mesquita7, Diogo Kuczera2, Juan C. Chachques1 and Katherine A. T. Carvalho8

1MSC-student*. Isolation and Culture Proceedings. Cell Therapy and Biotechnology in Regenerative Medicine Department, The Pelé Pequeno Príncipe Institute, Child and Adolescent Health Research, Pequeno Príncipe Faculty, Av. Silva Jardim 1632, Box 80.250-200, Curitiba, Paraná, Brazil
2Bioprocess Engineering and Biotechnology Department, Federal University of Paraná, Av. Coronel. Francisco Heráclito dos Santos 210, Box 81.531-970, Curitiba, Paraná, Brazil
3PhD-student*. Papanicolaou Staining. Cell Therapy and Biotechnology in Regenerative Medicine Department, The Pelé Pequeno Príncipe Institute, Child and Adolescent Health Research, Pequeno Príncipe Faculty, Av. Silva Jardim 1632, Box 80.250-200, Curitiba, Paraná, Brazil
4PhD student. Literature Reviewer. Federal University of Technology, Cristo Rei 19, Box 85.002-490, Toledo, Paraná, Brazil
5Professor, MD and PhD. Discussion. Laboratory of Biosurgical Research, Pompidou Hospital, University of Paris Descartes, Rue Leblanc, 20- Box 75015, Paris, France.
6Professor, MD and PhD. Citometric Analysis. Cell Therapy and Biotechnology in Regenerative Medicine Department, The Pelé Pequeno Príncipe Institute, Child and Adolescent Health Research, Pequeno Príncipe Faculty, Av. Silva Jardim 1632, Box 80.250-200, Curitiba, Paraná, Brazil
7PhD-student*. Bioprocess Engineering and Biotechnology Department, Federal University of Paraná, Av. Coronel. Francisco Heráclito dos Santos 210, Box 81.531-970, Curitiba, Paraná, Brazil
8Professor, MD and PhD. Discussion. Cardiovascular Surgery Department, Costantini Hospital, Rua Rosa Kaini Nadlov 190, Box 81.200-290, Curitiba, Paraná, Brazil
9Professor, MD, Citometric Analysis. Hospital de Clínicas, Haematology Department, Federal University of Paraná, Av. General Carneiro, 181 - Box 85.002-490, Curitiba, Paraná, Brazil
10PhD-student. Citometric Analysis. Hospital de Clínicas, Haematology Department, Federal University of Paraná, Av. General Carneiro, 181 - Box 85.002-490, Curitiba, Paraná, Brazil
11MD. Pathological Analysis. Pathology Department, Santa Casa de Misericórdia Hospital, Praça Rui Barbosa 694, Box 80.010-030, Curitiba, Paraná, Brazil
12PhD. Citometric Analysis. Cell Biology Department, Federal University of Paraná, Av. Coronel. Francisco Heráclito dos Santos 210, Box 81.531-970, Curitiba, Paraná, Brazil
13PhD-student. Literature Reviewer. Federal University of Technology, Cristo Rei 19, Box 85.002-490, Toledo, Paraná, Brazil
14Professor, MD and PhD. Experimental design, conclusion as writer of manuscript. Cell Therapy and Biotechnology in Regenerative Medicine Department, The Pelé Pequeno Príncipe Institute, Child and Adolescent Health Research, Pequeno Príncipe Faculty, Av. Silva Jardim 1632, Box 80.250-200, Curitiba, Paraná, Brazil

Abstract

The possibility of cell modifications compromises the safety of stem cell therapy under standardized conditions. The aim of this study was to analyze adipose tissue-derived mesenchymal stem cells (AT-MSCs) using the Pap test as a first screening step to evaluate genetic stability.

Methods: Human adipose tissue from six healthy female donors was obtained from elective liposuction procedures. The cells were isolated, cultivated at P2/P3, characterized by flow cytometric analysis, and differentiation induced. The AT-MSCs were stained by Papanicolaou (Pap) staining and analyzed according to the Bethesda classification, and viability-apoptosis relationships were evaluated.

Results: The Pap test for Sample I indicated high-grade alterations consistent with genetic instability; for Samples II-V, atypical cells of undetermined significance; and for Sample VI, normal cells.

Conclusions: These results demonstrate the potential of using the Pap test as an initial screening step to evaluate the genetic stability of cultured AT-MSCs as well for other adherent stem cells.

Keywords: Cultured; Mesenchymal stem cells; Therapy; Genetic stability; Human; Adipose-tissue; Papanicolaou; Safe

Introduction

The promise of stem cell-based therapy for advancement of research in regenerative medicine has stimulated a great number of clinical trials, particularly for previously untreatable diseases.

However, for new therapies, it is necessary to ensure the safety of processes and products in an efficient manner. Toward this aim, therapeutic use of biological products mandates the establishment and use of basic requirements known as Good Tissue Practices to prevent disease transmission, mix-ups, and cross-contamination, assuring safe cell therapy [1].

As with oncohematological diseases, cell-based regenerative therapy is expected to be useful in non hematological diseases with cells of adult origin, such as mesenchymal stem cells (MSCs) and induced pluripotent stem cells (iPSCs); both cell types are self-renewing

*Corresponding author: Katherine Alhayde Teixeira de Carvalho, MD, PhD. The Pele Pequeno Príncipe Institute, Child and Adolescent Health Research, Pequeno Príncipe Faculty, Av. Silva Jardim 1632, Box 80.250-200, Curitiba, Paraná, Brazil, Tel. 0055-41-3310-1719, Fax. 0055-41-32331699, E-mail: katherinecarv@gmail. com

Received August 28, 2011; Accepted October 17, 2011; Published October 19, 2011

Citation: Irioda AC, Zocche L, Souza CMCO, Ferreira RJ, Aliprandini E, et al. (2011) Pap Test as the First Step in Screening Genetic Stability in Cell-Based Therapy. J Stem Cell Res Ther 1:106. doi:10.4172/2157-7633.1000106

Copyright: © 2011 Irioda AC, et al. This is an open-access article distributed under the terms of the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original author and source are credited.
populations and have pluripotent capacities to regenerate various tissues: cartilage, bone, muscle tendons, and the nervous system [2,3]. MSCs could also be used for transplantation in cell-based therapy, and for this purpose, must be able to expand ex vivo undercultivation. They should be able to grow in flasks based in their characteristic of adherence to plastic. However, the literature has demonstrated that transplantation is limited by the number of MSCs; thus, better results are dependent on producing a great number of cells independent of their source and consequently, large numbers of cells are usually required for clinical applications. Transplantation is also limited by mortality of the transplanted cells after transplantation by apoptosis or macrophage activity independent of the autologous or allogeneic procedures used. For the same reasons, iPSCs must also undergo expansion under ex-vivo conditions.

These considerations highlight the need for the in vitro expansion of stem cells prior to their commitment into tissue-specific applications. Before bioreactors can be used, several factors must be considered, including the need to increase the number of cells, the capacity to support high cell densities in relatively small volumes, the ability to scale up the design, and standardization of the bioprocess, including the safety of the products [4].

Difficulties have been reported during cultivation of MSCs for expansion. Spontaneous transformation of murine hematopoietic MSCs (mMSCs) has been described by many authors, although there are contradictory results [5,6]. However, another group observed spontaneous malignant transformation in a preclinical study in which the cells had modified characteristics after the third passage. Direct injection of cultured bone marrow-derived MSCs into immunocompetent mice has resulted in tumor formation, and a karyotype analysis showed that increased chromosome numbers and multiple Robertsonian translocations occurred at passage 3 coincident with the loss of contact inhibition [7,8]. On the other hand, spontaneous transformation over the long-term in human MSCs (hMSCs) from bone marrow cultures has not been reported in other studies [9,10]. In humans, one group observed spontaneous malignant transformation of adipose tissue-derived (AT)-MSCs in vitro [11].

Ultimately, Røsland et al. [12] conclusively demonstrated spontaneous malignant transformations in long-term cultured bone marrow-derived human MSCs (5-106 weeks). The possibility of cell modifications compromises the reproducibility and safety of generation of MSCs under standardized conditions. The consequence of these results was increased requirements to maintain safe cell therapy beyond the basic requirements and regulations of government health organizations to ensure the identity, purity, potency, and clinical efficacy of MSCs.

The protocol governs quality control of cells by the FDA, excluding genetically modified cells, and primarily addresses safety concerns with cell differentiation to undesired cell types and potential uncontrolled cell proliferation or tumorigenicity [13]. Others concerns about the safety of this type of therapy are related to the stability of the product and/or the cell line, specifically, the number of passages/doublings over time, and maintaining desired differentiation properties without karyotypic alterations [14,15].

All of those proceedings impose great costs in translating these therapies to humans and barriers to democratization of advanced therapy approaches. If cell-based therapies could be made more efficient, more people could access their benefits; ideally, the cost of each step would be reduced, without prejudicing the safety of the therapy.

In our laboratory, over the last eight years, we have made morphological observations of undifferentiated MSCs from human bone marrow and adipose tissue up to P5 (not for transplantation use), reviewing and recording a diary of cultured cells using an inverted microscope for the purposes of research academics. We have observed some issues that would be of concern regarding the health of cultured MSCs for potential use in transplantation cell-based therapy. In developing countries, particularly in Brazil where our group is established, we have limited financial resources and at times, we need to answer certain questions without great cost. Why not “do simple things in a simple way” [16]?

With that concept in mind, we chose the Papanicolaou test, known as the Pap smear, a cytological analysis of exfoliated cells from the cervix originally described by George Papanicolaou in 1928 [17]. This test is efficient in diagnosing early abnormal changes in the cervical epithelium that could indicate the presence of cervical cancer, and is universally used for screening for cervical cancer in women [18].

The possibility of analyzing cultured MSCs at low cost using the Pap test appears very attractive to avoid additional steps, as a first step why it is a simple and short procedure requiring laboratory equipment available to everyone, in contrast to the more cumbersome trial procedures required by the FDA, if, similar abnormalities as for squamous cells of the cervix can be demonstrated. The aim of this study was to analyze AT-MSCs using the Pap test.

Materials and Methods

Isolation of adipose tissue-derived MSCs

Human abdominal AT from six healthy female donors was obtained and designated Samples 1-VI (ages 47, 38, 24, 45, 35, and 41 years with body-mass index 22.3, 29.4, 24.7, 29.4, 22.8, and 23.6, respectively). Samples were obtained during elective liposuction procedures for plastic and cosmetic purposes under local anesthesia after informed consent, following the guidelines of the Brazilian Ethics Committee on Use of Human Subjects and as approved by the Pequeno Príncipe Hospital Complex Ethics Committee, number 0617-08.

The AT-MSCs were isolated in accordance with Zuk et al. (2001) [19]. The liposapirates were washed extensively with sterile PBS to remove contaminating debris and red blood cells. The washed aspirates were treated with 0.075% collagenase (type I, Sigma, USA) in PBS for 30 minutes at 37°C with gentle agitation. The collagenase was inactivated with an equal volume of DMEM/10% FBS (Gibco BRL, Life Technologies, Inc Grand Island, NY) and the pellet was centrifuged for 10 minutes at low speed before being resuspended in DMEM/F12/10% FBS with 1% antibiotic (streptomycin- penicillin; Gibco BRL, Life Technologies, Inc, Grand Island, NY, USA), and filtered through a 100-μm mesh filter to remove debris. The filtrate was centrifuged and plated onto conditioned tissue culture plates and the samples were then characterized by flow cytometric analysis.

Cell characterization and viability-apoptosis relationships

Flow cytometric analysis (FACScalibur; Becton Dickinson) was performed to validate the MSCs from adipose tissue. The harvested cells from the culture dishes underwent flow cytometric analysis to
characterize the immunophenotypic profile of cellular subsets using the surface markers CD45, CD34, CD49d, CD73, CD90, and CD105.

Viability-apoptosis relationships were characterized using conjugated 7AD-annexin. The procedure used was “stain then lyse” lysis was performed after staining with monoclonal antibodies (MAb). The cells were incubated with the specific MAb panel for 15 minutes at room temperature in the dark, lysed (FACS lysing solution, Becton Dickinson), washed with 0.1% PBS, resuspended, and analyzed on a FACS calibur using Cell Quest software (Becton Dickinson).

For the 4-color staining immunophenotyping analyses, the following rat antibody conjugates, coupled with fluorescein isothiocyanate (FITC), phycoerythrin (PE), CyChrome (PE-Cy5), or allophycocyanin (APC) were used: CD45-PE-Cy5 (clone HI30), CD34-FITC (clone 581), CD49d-PE (clone 9F10), CD105-FITC (clone 266), CD73-PE (clone AD2), and CD90-FITC (clone 5E10). All antibodies were used at the concentrations recommended by the manufacturer. The respective isotype-matched control was PE MoAb.

### Induced differentiation

Differentiated AT-MSCs were processed as described in Zuk et al. [20]. Briefly, the AT-MSCs were washed extensively with sterile PBS to remove contaminating debris and were cultured in induction medium DMEM/F12 and 10% FBS supplemented with the growth factors for each cell type: for adipogenic, 0.01 Mm 1,25-dihydroxyvitamin D3, 50 μM ascorbate-2-phosphate, 10 mM β-glycerophosphate, and 1% antibiotic (streptomycin-penicillin); and for osteogenic, 0.5 mM isobutyl-methylxanthine, 1 μM dexamethasone, 10 μM insulin, 200 μM indomethacin, and 1% antibiotic (streptomycin-penicillin).

### Cytology and immunocytochemistry

Induced AT-MSCs were stained for the assays with Alizarin Red for osteogenesis and Sudan Red for adipogenesis.

### Papanicolaou staining

The Pap test is based on specific changes in the cytoplasm and/or nucleus of the epithelial cells of the cervix, which can be identified through light microscopy. A combination of two coloring agents is used: a nuclear dye, Harris hematoxylin; and a mixture of two cytoplasmatic dyes, orange G and polychrome mixture (EA51) highlights the cellular aspects that are fundamental in differentiating normal squamous cells of the cervix from low- and high-grade squamous intraepithelial lesions (LSILs and HSILs, respectively) [21,22]. These aspects include nuclear pigmentation, position, volume, and morphology; membrane regularity and integrity; and the chromatin arrangement. Other important parameters are the cellular size and shape, and the ratio of the nucleus to the cytoplasm, which is increased in pre-cancerous cells.
In this study, the AT-MSCs were seeded in 25-cm² flasks at 5 × 10⁴/mL, then cultured for 7-14 days to P2 and P3 until 70% confluence was reached. The cells were then cultured for three days on double-chamber slides, after which the cultures were ended and the stain applied directly to the slides. Undifferentiated AT-MSCs were stained by Papanicolaou staining. Each slide was immersed in vats containing the staining solutions in sequence for the appropriate immersion times in accordance with the manufacturer’s instructions (Newprov, Pinhais, Brazil). The slides were then mounted and observed under a light microscope. The evaluation of PAP-stained cells have been done by three independent pathologists and the result was determined in accordance with the Bethesda classification as (a) normal cells, (b) atypical cells of undetermined significance, or (c) high-grade alterations consistent with genetic instability [22,24].

Results

Figure 1 shows the results of the flow cytometric analysis for Samples I, as well the viability/apoptosis relationships (Table 1). The obtained cells were demonstrated to be MSCs from adipose tissue why the cells were marked with antibodies that are in consensus of adipose origin as well the cells were integrity preservation demonstrates by great viability and small apoptosis fraction; all samples have demonstrated the normal profile of undifferentiated adipose derived stem cells, the cells were adherents and all samples were capable of differentiation in lipid cells demonstrated by oil Sudan red and bone by alizarin osseous matrix identification, respectively (Figure 2). The Pap test for Sample I showed high-grade alterations consistent with genetic instability (Figure 3), Samples II-V contained atypical cells of undetermined significance (Figure 4a, Figure 4b) and Sample VI comprised normal cells (Figure 4c, Figure 4d).

Discussion

These results demonstrate the potential for using the Pap test as the initial step of a screening methodology to ensure the genetic stability of cultured MSCs. As described by various authors, the difficulty in assuring accuracy in the Pap test is more in the preparation of cervical smears than in the analysis, because it is possible to use rescreening methods for negative results [23,25,26].

It is much easier to obtain a thin and clean smear starting from a cell culture than from the cervical samples normally used in a conventional Pap test. The Pap test is used to differentiate cells in many types of specimens: cerebrospinal, abdominal, or pleural fluids; synovial tissues; tumor samples; or other materials containing cells [23,25,26]. The cytopathological findings for the AT-MSC samples in this
The AT is “aged” - the differentiated adipocytes as well their quiescent AT-MSCs and these cells are likely at increased risk of genetic instability. The adult MSCs were demonstrated to differentiate in varying concentrations in both solid and hematopoietic tissues in blood suspension; however, while the pluripotentiality of AT-MSCs may be similar to those of hematopoietic origin, their genetic stability may not be [29,30]. The abnormality findings in a previous study of AT-MSCs suggest that inclusion of the Pap test for both allogeneic and autologous donors would be valuable, because abnormalities may develop in cultured cells independent of the type of donor [31].

These finding suggests that the Pap test could be used not only for adult sources, but also for all adherent cells from embryos, fetuses, and adult tissue such as iPSCs or MSCs. The results of our study demonstrate the usefulness of the Pap test as the initial quality control step for cultured stem cell therapy, independent of the origin, source, or type of transplantation. This approach seems reasonable if identification of modified cells in this first step could avoid additional costs. If modified cells were present at this first step, they would not be considered safe for use in transplantation. The atypical cells and cells of unknown significance are not safe; they may not be pathogenic, but they may transform to cancer stem cells. Caution is required and the risk is not considered acceptable. If there were no modified cells according to the Pap test results, the next step of the screening procedure involving the karyotype analysis would be followed.

Tsuji et al. [32] has suggested with respect to iPSCs transplantation that to await the results of injecting cells into mice and subsequent tumor development to exclude cells for transplantation after two months will increase the delay in deciding whether to use the cells. The reprogramming process and subsequent culture of iPSCs in vitro can induce genetic and epigenetic abnormalities as well as the human embryonic stem cells that could acquire an abnormal number of chromosomes. They justify the caution with cultured stem cell therapy [33].

On the other hand, we discuss the impact of concentration of FBS could be responsible for “fibroblastic” transformation of AT-MSC (making them potentially more tumorigenic) as the concentration of the 10% Fetal Bovine Serum-containing media used to expand cells and whether this Lower FBS media might reduce the cellular abnormalities seen during expansion as the cells would divide less rapidly [34]. We concur, and further suggest that our results support the proposition to introduce the Pap test into the algorithm for screening cell modification as an initial step (Figure 5). To contribute for the usefulness of this test as a screening step is the possibilities to exclude in the start of screening the cells that have definite and indubitable high-grade alterations.
Acknowledgments

*Financial support was received from Coordination for the Improvement of Higher Level - or Education- Personnel (CAPES) for Post-Graduation Student Grants.

References

1. Marwaha N, Saluja K, Sharma RR (2007) Stem cell therapy: the need for current Good Manufacturing Practices. Bull PGI 41: 49-51.
2. Florian MC, Geiger H (2010) Concise review: polarity stem cells, disease, and aging. Stem Cells 28: 1623-1629.
3. Das AK, Pal R (2010) Induced pluripotent stem cells (iPSCs): the emergence of a new champion in stem cell technology-driven biomedical applications. J Tissue Eng Regen Med 4: 413-421.
4. Placzek MR, Chung IM, Macedo HM, Mortera Blanco T, et al. (2009) Stem cell bioprocessing: fundamentals and principles. J R Soc Interface 6: 209-232.
5. Zhou YF, Bosch-Maroé M, Okuyama H, Krishnamachary B, Kimura H, et al. (2006) Spontaneous aneuploidy of mouse bone marrow-derived stromal cells. Cancer Res 66: 10849-10854.
6. Li H, Fan X, Kovi RC, Jo Y, Moquin B, et al. (2007) Spontaneous expression of embryonic factors and p53 point mutations in aged mesenchymal stem cells: a model of age-related tumorigenesis in mice. Cancer Res 67: 10889-10898.
7. Rubin D, Garcia-Castro J, Martin MC, de la Fuente R, Cigudosa JC, et al. (2006) Spontaneous human adult stem cell transformation. Cancer Res 66: 10849-10854.
8. Bapat SA, Mali AM, Koppkar CB, Kurrey N (2005) Stem and progenitor-like cells contribute to the aggressive behavior of human epithelial ovarian cancer. Cancer Res 65: 3025-3029.
9. Bernardo ME, Zaffaroni N, Novara F, Cometa AM, Avanzini MA, et al. (2007) Human bone marrow-derived mesenchymal stem cells do not undergo transformation after long-term in vitro culture and do not exhibit telomere maintenance mechanisms. Cancer Res 67: 9142-9149.
10. Izadpanah R, Kaushal D, Kriedt C, Tsien F, Patel B, et al. (2008) Long-term in vitro expansion alters the biology of adult mesenchymal stem cells. Cancer Res 68: 4229-4238.
11. Meza-Zepeda LA, Noer A, Dahl JA, Micco F, Myklebost O, et al. (2008) High-resolution analysis of genetic stability of human adipose tissue stem cells cultured to senescence. J Cell Mol Med 12: 553-563.
12. Rasland GV, Svedensén A, Torsvik A, Sobala E, McCormack, et al. (2009) Long-term cultures of bone marrow-derived human mesenchymal stem cells frequently undergo spontaneous malignant transformation. Cancer Res 69: 5331-5339.
13. Halme DG, Kessler DA (2006) FDA regulation of stem-cell-based therapies. N Engl J Med 355: 1730-1736.
14. Condie ML, Rao M (2008) Regulatory issues for personalized pluripotent stem cells. Stem Cells 26: 2753-2758.
15. National Institute of Mental Health; University of Virginia. In: ClinicalTrials.gov [Internet]. Bethesda (MD): National Library of Medicine (USA) [cited 2010 July 17].
16. Shakespeare W (1975) The Complete Works of William Shakespeare - Henry IV. MCMXCV by Crown Publishers, Inc, United States of America.
17. Shingleton HM, Patrick RL, Johnston WW, Smith RA (1995) The current status of the Papanicolaou smear. CA Cancer J Clin 45: 305-320.
18. Wallin KL, Wiktund F, Angstöm T, Bergman F, Stendahl U, et al. (1999) Type-specific persistence of human papillomavirus DNA before the development of invasive cervical cancer. N Engl J Med 341: 1633-1638.
19. Zuk PA, Zhu M, Mizuno H, Huang J, Futrell JW, et al. (2001) Multilineage cells from human adipose tissue: implications for cell-based therapies. Tissue Eng 7: 211-228.
20. Zuk PA, Zhu M, Ashjian P, De Ugarte DA, Huang Ji, et al. (2002) Human adipose tissue is a source of multipotent stem cells. Mol Biol Cell. 13: 4279-4295.
21. Muntean M (2009) Cytological identification of the (pre)cancerous cervical lesions within a clinically asymptomatic female population. Curr Health Sci J 35: 176-179.
22. National Cancer Institute Workshop (1989) The 1988 Bethesda System for reporting cervical/vaginal cytological diagnoses. JAMA 262: 931-934.
23. Nanda K, McCrory DC, Myers ER, Bastian LA, Hasselblad V, et al. (2000) Accuracy of the Papanicolaou test in screening for and follow-up of cervical cytologic abnormalities: a systematic review. Ann Intern Med 132: 810-819.
24. Aggar BS, Zoschnick L, Wright JTC (2003) Cytology terminology. Am Fam Physician 68: 1992-1998.
25. Tavares SB, Alves de sousa NL, Manrique EJ, Pinheiro de Albuquerque ZB, Zeffelino LC, et al. (2008) Comparison of the performance of rapid prescreening, 10% random review, and clinical risk criteria as methods of internal quality control in cervical cytology. Cancer 25: 165-170.
26. Utagawa ML, Shirata NK, Mattosinho de Castro FJM, di Loreto C, Dalfagnol M, et al. (2010) Performance of 3 methods for quality control for gynecologic cytology diagnoses. Acta Cytol 52: 439-444.
27. Freter R, Osawa M, Nishikawa S (2010) Adult stem cells exhibit global suppression of RNA polymerase II serine-2 phosphorylation. Stem Cells 28: 1571-1589.
28. Izadpanah R, Kaushal D, Kriedt C, Tsien F, Patel B, et al. (2008) Long-term in vitro expansion alters the biology of adult mesenchymal stem cells. Cancer Res 68: 4229-4238.
29. Bochkoiv NP, Nikitina VA, Buyanovskaya OA, Voronina ES, Goldstein DV, et al. (2008) Aneuploidy of stem cells isolated from human adipose tissue. B Exp Biol Med 146: 344-347.
30. Buyanovskaya OA, Kuleshov NP, Nikitina VA, Voronina ES, Katsova LD, et al. (2009) Spontaneous aneuploidy and clone formation in adipose tissue stem cells during different periods of culturing. B Exp Biol Med 148: 109-112.
31. Choumerianou DM, Dimitriou H, Perdikogianni C, Martimianaki G, Rinimucci M, et al. (2008) Study of oncogenic transformation in ex vivo expanded mesenchymal cells, from paediatric bone marrow. Cell Prog 41: 909-922.
32. Tsui O, Miura K, Okada Y, Fujiyoshi K, Mukaino M, et al. (2010) Therapeutic potential of appropriately evaluated safe-induced pluripotent stem cells for spinal cord injury. Proc Natl Acad Sci 107: 12704-12709.
33. Hussein SM, Batada NN, Vuoristo S, Ching RW, Auto R, et al. (2011) Copy number variation and selection during reprogramming to pluripotency. Nature 47: 56-62.
34. Karahuseynoglu S, Cinar O, Kılıc E, Kara F, Akay GG, et al. (2007) Biology of Stem Cells in Human Umbilical Cord Stromata: In Situ and In Vitro Surveys. Stem Cell Res Ther 1: 1000106. doi:10.4172/2157-7633.1000106