

- Ozkinay C., Mitelman F. 1979. A simple trypsin-Giemsa technique producing simultaneous G- and C-banding in human chromosomes. *Hereditas*. 90 : 1—4.
- Park S. B., Seo K. W., So A. Y., Seo M. S., Yu K. R., Kang S. K., Kang K. S. 2012. SOX2 has a crucial role in the lineage determination and proliferation of mesenchymal stem cells through Dickkopf-1 and c-MYC. *Cell Death Differ*. 19 : 534—545.
- Poljanskaya G. G., Vakhtin Y. B. 2003. The karyotypic structure of cell populations *in vitro* as integral system. *Tsitolgiya*. 45 (2) : 115—131.
- Redaelli S., Bentivegna A., Foudah D., Miloso M., Redondo J., Riva G., Baronchelli S., Dalpra L., Tredici G. 2012. From cytogenomic to epigenomic profiles: monitoring the biologic behavior of *in vitro* cultured human bone marrow mesenchymal stem cells. *Stem Cell Res. Ther.* 3 : 47—63.
- Ren H., Sang Y., Zhang F., Liu Z., Qi N., Chen Y. 2016. Comparative analysis of human mesenchymal stem cells from umbilical cord, dental pulp, and menstrual blood as sources for cell therapy. *Stem Cells Int.* Doi: 10.1155/2016/3516574.
- Reyes M., Lund T., Lenvik T., Aguiar D., Koodie L., Verfaillie C. M. 2001. Purification and *ex vivo* expansion of postnatal human marrow mesodermal progenitor cells. *Blood*. 98 : 2615—2625.
- Riekstina U., Cakstina I., Parfejevs V., Hoogduijn M., Jankovskis G., Muiznieks I., Muceniece R., Ancans J. 2009. Embryonic stem cell marker expression pattern in human mesenchymal stem cells derived from bone marrow, adipose tissue, heart and dermis. *Stem Cell Rev.* 5 : 378—386.
- Sarugaser R., Hanoun L., Keating A., Stanford W. L., Davies J. E. 2009. Human mesenchymal stem cells self-renew and differentiate according to a deterministic hierarchy. *PLoS ONE*. 4 : e6498. Doi: 10.1371/journal.pone.0006498.
- Savickiene J., Baronaite S., Zentelyte A., Treigyte G., Navakauskiene R. 2016. Senescence-associated molecular and epigenetic alterations in mesenchymal stem cell cultures from amniotic fluid of normal and fetus-affected pregnancy. *Stem Cells Int.* 2016 : 2019498.
- Senseb   L., Krampera M., Schrezenmeier H., Bourin P., Giordano R. 2010. Mesenchymal stem cells for clinical application. *Vox Sang.* 98 : 93—107.
- Sethe S., Scutt A., Stolzing A. 2006. Aging of mesenchymal stem cells. *Ageing Res. Rev.* 5 : 91—116.
- Shaffer I. G., Slovack M. L., Campbell L. J. (Eds.). 2009. An international system for human cytogenetic nomenclature. Basel: S. Karger. 138 p.
- Shih D. T., Lee D. C., Chen S. C., Tsai R. Y., Huang C. T., Tsai C. C., Shen E. Y., Chiu W. T. 2005. Isolation and characterization of neurogenic mesenchymal stem cells in human scalp tissue. *Stem Cells*. 23 : 1012—1020.
- Siegel G., Kluba T., Hermanutz-Klein U., Bieback K., Northoff H., Schafer R. 2013. Phenotype, donor age and gender affect function of human bone marrow-derived mesenchymal stromal cells. *BMC Med.* 11 : 146. Doi: 10.1186/1741-7015-11-146.
- Sonoyama W., Liu Y., Yamaza T., Tuan R. S., Wang S., Shi S., Huang G. T. 2008. Characterization of the apical papilla and its residing stem cells from human immature permanent teeth: a pilot study. *J. Endod.* 34 : 166—171.
- Suchanek J., Nasry S. A., Soukup T. 2017. The differentiation potential of human natal dental pulp stem cells into insulin-producing cells. *Folia Biol. (Praha)*. 63 : 132—138.
- Szepesi  , Matula Z., Szigeti A., V  rady G., Szalma J., Szab   G., Uher F., Sarkadi B., N  met K. 2016. *In vitro* characterization of human mesenchymal stem cells isolated from different tissues with a potential to promote complex bone regeneration. *Stem Cells Int.* 2016 : 3595941. Doi: 10.1155/2016/3595941.
- Tobin D. J. 2017. Introduction to skin aging. *J. Tissue Viability*. 26 : 37—46.
- Ullah I., Park J. M., Kang Y. H., Byun J. H., Kim D. G., Kim J. H., Kang D. H., Rho G. J., Park B. W. 2017. Transplantation of human dental pulp-derived stem cells or differentiated neuronal cells from human dental pulp-derived stem cells identically enhances regeneration of the injured peripheral nerve. *Stem Cells Develop.* 26 : 1247—1257.
- Wagner W., Horn P., Castoldi M., Diehlmann A., Bork S., Saffrich R., Benes V., Blake J., Pfister S., Eckstein V., Ho A. D. 2008. Replicative senescence of mesenchymal stem cells: a continuous and organized process. *PLoS ONE*. 3 : e2213. Doi: 10.1371/journal.pone.0002213.
- Wang H., Zhong Q., Yang T., Qi Y., Fu M., Yang X., Qiao L., Ling Q., Liu S., Zhao Y. 2018. Comparative characterization of SHED and DPSCs during extended cultivation *in vitro*. *Mol. Med. Rep.* 17 : 6551—6559.
- Wu R., Gu B., Zhao X., Tan Z., Chen L., Zhu J., Zhang M. 2013. Derivation of multipotent nestin(+)CD271(−)/STRO-1(−) mesenchymal-like precursors from human embryonic stem cells in chemically defined conditions. *Hum. Cell*. 26 : 19—27.
- Xiao L., Kumazawa Y., Okamura H. 2014. Cell death, cavitation and spontaneous multi-differentiation of dental pulp stem cells-derived spheroids *in vitro*: a journey to survival and organogenesis. *Biol. Cell*. 106 : 405—419.
- Yan Z. J., Hu Y. Q., Zhang H. T., Zhang P., Xiao Z. Y., Sun X. L., Cai Y. Q., Hu C. C., Xu R. X. 2013. Comparison of the neural differentiation potential of human mesenchymal stem cells from amniotic fluid and adult bone marrow. *Cell Mol. Neurobiol.* 33 : 465—475.
- Yi Q., Liu O., Yan F., Lin X., Diao S., Wang L., Jin L., Wang S., Lu Y., Fan Z. 2017. Analysis of senescence-related differentiation potentials and gene expression profiles in human dental pulp stem cells. *Cells Tissues Organs*. 203 : 1—11.
- Zhang M., Wang Z., Zhao Y., Zhang L., Xu L., Cao L., He W. 2018. The effect of age on the regenerative potential of human eyelid adipose-derived stem cells. *Stem Cells Int.* 2018 : 5654917. Doi: 10.1155/2018/5654917.
- Zhang N., Lu X., Wu S., Li X., Duan J., Chen C., Wang W., Song H., Tong J., Li S., Liu Y., Kang X., Wang X., Han F. 2018. Intrastriatal transplantation of stem cells from human exfoliated deciduous teeth reduces motor defects in Parkinsonian rats. *Cyotherapy*. Pii: S1465-3249(18)30407-9. Doi: 10.1016/j.jcyt.2018.02.371.
- Zhang W., Walboomers X. F., Shi S., Fan M., Jansen J. A. 2006. Multilineage differentiation potential of stem cells derived from human dental pulp after cryopreservation. *Tissue Eng.* 12 : 2813—2823.

Поступила 5 VI 2018

## THE DERIVATION AND CHARACTERIZATION OF MESENCHYMAL STEM CELL LINE, ISOLATED FROM HUMAN PULP OF A DECIDUOUS TOOTH

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The derived and characterized the new non-immortalized human mesenchymal stem cell lines isolated from pulp of a deciduous tooth of a child, named MSC-DP. The analysis of the main characteristics was carried out on 6<sup>th</sup> and 25<sup>th</sup> passages. In the process of long-term cultivation gradually increases the proportion of senes-

cence cells according to the activity of  $\beta$ -galactosidase. The 25th passage significantly increases the expression of the CD264 marker in comparison with the earlier passages, the increased level of which also characterizes cells that have entered the active phase of replicative senescence. The plating efficiency of cells of MSC-DP significantly decreases during long-term cultivation. Growth curves indicate active cell line proliferation on the 6<sup>th</sup> passage and a significant decrease in the proliferation index on the 25<sup>th</sup> passage. Karyotypic analysis carried out on the 6<sup>th</sup> and 25<sup>th</sup> passages showed the presence of normal human karyotype, 46, XX. Significant differences in karyotypic structure between 6<sup>th</sup> and 25<sup>th</sup> passage associated with the presence of a significant number dicentrics chromosome-type telomere associations in the later passage. The predominant participation in the formation of telomeric associations of the long arm of the chromosome 14 was found. On the 6<sup>th</sup> and 25<sup>th</sup> passages the expression of surface antigens typical for human MSCS (CD44, CD73, CD90, CD105, HLA-ABC) and the absence of expression of CD34, CD45, HLA-DR were revealed. The marker of undifferentiated human embryonic stem cells (ESC) — SSEA-4 is expressed only on the 6<sup>th</sup> passage. The expression of markers of early differentiation ESC in the derivatives of the 3 germ layers was shown for cells of MSC-DP line. Cells of the MSC-DP line have the ability to differentiate in the osteogenic and chondrogenic directions. Expression of the neuronal differentiation gene is shown in confirmation of the ability of the MSC-DP line to multipotent differentiation. Overall, the presented results confirm the status of MSCs for the derived line and indicate significant changes occurring in the process of replicative senescence.

**Key words:** human mesenchymal stem cells, proliferation, replicative senescence, surface cell markers, karyotype, telomeric associations, differentiation