

МОНГОЛ УЛСЫН БОЛОВСРОЛ, СОЁЛ ШИНЖЛЭХ УХААН,
СПОРТЫН ЯАМ
АНАГААХЫН ШИНЖЛЭХ УХААНЫ ҮНДЭСНИЙ ИХ СУРГУУЛЬ

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ЭНХБАЯРЫН БАЯРМАА

**ХУЛГАНЫ ЯСНЫ ХЭМЭЭС ЯЛГАН АВСАН ҮҮДЭЛ ЭСИЙГ
ХҮЧИЛТӨРӨГЧИЙН ЯЛГААТАЙ ОРЧИНД ӨСГӨВӨРЛӨСӨН ДҮН**

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Улаанбаатар хот

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Culture mouse bone marrow derived stem cells under hypoxic conditions

Key word: colony forming unit, colony, mesenchymal stem cell, oxygen concentration, cell marker

Background: The use of stem cells of various clinical applications is highly expected and the production of good quality stem cells is very critical for basic studies. In the bone marrow, hematopoietic and mesenchymal stem cells from an unique niche in which the oxygen tension is low. Hypoxia may have a role in maintaining stem cell fate, self renewal and multi-potency. We investigated whether low oxygen culture would be beneficial for hematopoietic stem cell and mesenchymal stem cell.

Material and methodology: BMCs from 8-12 week aged mice were subjected to hypoxic conditioning by culture for 8-10 days in 20%, 3%, 1% oxygen. For culture 2×10^5 cell/ml were seeded in colony forming assay and 2×10^6 cell/ml were seeded in chamber slide. We counted cell colonies under different hypoxic conditions by Olympus IX71 fluorescence microscope. After cell culture in chamber slide, we stained cells by anti-CD90 and anti-CD105 then counted positive cells by Olympus IX71 fluorescence microscope.

Results: Compared to normoxic cells and hypoxic cells well morphologically undifferentiated and counted by Olympus IX71 microscope. More colonies and cell markers were observed at 3%, 1% oxygen. Statistical significances were identified with granulocytes and macrophage colony ($p < 0.05$) in hypoxic condition.

Conclusions: Our data suggests low physiological oxygen culture could improve the stemness of macrophage and granulocytes colony and improve the differentiation of mesenchymal cells. Long term culture will be necessary to confirm whether low physiological oxygen levels also improve genomic stability.

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1. Koch Tg BL, Betts DH. Current and future regenerative medicine-principles, concepts, and therapeutic use of stem cell therapy and tissue engineering in equine medicine. *Can Vet J*. 2009;50(2):155-165.
2. Baksh D SL TR. Adult mesenchymal stem cells: characterization, differentiation, and application in cell and gene therapy. *J Cell Mol Med*. 2004(8):301-316.
3. McCulloch EA SL, Till JE, Russell ES, Bernstein SE The cellular basis of the genetically determined hemopoietic defect in anemic mice of genotype Sl-Sld. *Blood*. 1965(26):399-410.
4. RS T. Blood and bone: two tissues whose fates are intertwined to create the hematopoietic stem cell niche. *Blood*. 2005(105):2631-2639.
5. Wilson A TB. Bone marrow haematopoietic stem cell niches. *Nat Rev Immunol* 2006(267):423-425.
6. Cipolleschi MG DSP, Olivotto M. The role of hypoxia in the maintenance of hematopoietic stem cells. *Blood*. 1993;82:2031-2037.
7. Malladi P XY, Chiou M, Giaccia AJ, Longaker MT Effect of reduced oxygen tension on chondrogenesis and osteogenesis. *Am J Physiol Cell Physiol*. 2006;290:139-146.
8. Salim A NR, Morgan EF, Giaccia AJ, Longaker MT. Transient changes in oxygen tension inhibit osteogenic differentiation and Run² expression in osteoblasts. *Biol Chem*. 2004;279:40007-40016
9. Grayson WL ZF, Izadpanah R, Bunnell B, Ma T. Effects of hypoxia on human mesenchymal stem cell expansion and plasticity in 3 D construct *J Cell Physiol*. 2007(207):331-339.
10. Guo CW, Kawakatsu M, Idemitsu M, et al. Culture under low physiological oxygen conditions improves the stemness and quality of induced pluripotent stem cells. *J Cell Physiol*. Nov 2013;228(11):2159-2166.
11. Suda T, Takubo K, Semenza GL. Metabolic regulation of hematopoietic stem cells in the hypoxic niche. *Cell Stem Cell*. Oct 04 2011;9(4):298-310.
12. Halfon S, Abramov N, Grinblat B, Ginis I. Markers distinguishing mesenchymal stem cells from fibroblasts are downregulated with passaging. *Stem Cells Dev*. Jan 2011;20(1):53-66.
13. Becker AJ, Mc CE, Till JE. Cytological demonstration of the clonal nature of spleen colonies derived from transplanted mouse marrow cells. *Nature*. Feb 2 1963;197:452-454.
14. Siminovitch L ME, Till JE. The distribution of colony-forming cells among spleen colonies. *J Cell Physiology*. 1963(62):9.
15. Nair V. Stem cell transplantation. *API medical update*. 2004;14:11.
16. Murrell W, Feron F, Wetzig A, et al. Multipotent stem cells from adult olfactory mucosa. *Dev Dyn*. Jun 2005;233(2):496-515.
17. Basciano L, Nemos C, Foliguet B, et al. Long term culture of mesenchymal stem cells in hypoxia promotes a genetic program maintaining their undifferentiated and multipotent status. *BMC cell biology*. 2011;12:12.
18. Sylvestar Darvin Sandhaanam GP, etc. Mesenchymal stem cells (MSC): Identification, Proliferation and Differentiation –A Review Article. *PeerJ Preprint*. 2013;1:7.
19. Niwa H MJ, Smith AG. Quantitative expression of Oct-3/4 defines differentiation, dedifferentiation or self-renewal of EC cells. *Nature Genetics*. 2000;24(4):6.

20. Cavaleri F, Scholer HR. Nanog: a new recruit to the embryonic stem cell orchestra. *Cell*. May 30 2003;113(5):551-552.
21. Wang X, Yang YJ, Jia YJ, et al. [The best site of transplantation of neural stem cells into brain in treatment of hypoxic-ischemic damage: experiment with newborn rats]. *Zhonghua Yi Xue Za Zhi*. Mar 27 2007;87(12):847-850.
22. Molofsky AV, Pardal R, Iwashita T, Park IK, Clarke MF, Morrison SJ. Bmi-1 dependence distinguishes neural stem cell self-renewal from progenitor proliferation. *Nature*. Oct 30 2003;425(6961):962-967.
23. Park IK, Qian D, Kiel M, et al. Bmi-1 is required for maintenance of adult self-renewing haematopoietic stem cells. *Nature*. May 15 2003;423(6937):302-305.
24. Dontu G, Jackson KW, McNicholas E, Kawamura MJ, Abdallah WM, Wicha MS. Role of Notch signaling in cell-fate determination of human mammary stem/progenitor cells. *Breast Cancer Res*. 2004;6(6):R605-615.
25. Beachy PA, Karhadkar SS, Berman DM. Tissue repair and stem cell renewal in carcinogenesis. *Nature*. Nov 18 2004;432(7015):324-331.
26. Marshall GP, 2nd, Laywell ED, Zheng T, Steindler DA, Scott EW. In vitro-derived "neural stem cells" function as neural progenitors without the capacity for self-renewal. *Stem Cells*. Mar 2006;24(3):731-738.
27. Shackleton M, Vaillant F, Simpson KJ, et al. Generation of a functional mammary gland from a single stem cell. *Nature*. Jan 5 2006;439(7072):84-88.
28. Short B, Brouard N, Occhiodoro-Scott T, Ramakrishnan A, Simmons PJ. Mesenchymal stem cells. *Arch Med Res*. Nov-Dec 2003;34(6):565-571.
29. Shooter RA, Gey GO. Studies of the mineral requirements of mammalian cells. *Br J Exp Pathol*. Feb 1952;33(1):98-103.
30. Braun RD, Lanzen JL, Snyder SA, Dewhirst MW. Comparison of tumor and normal tissue oxygen tension measurements using OxyLite or microelectrodes in rodents. *Am J Physiol Heart Circ Physiol*. Jun 2001;280(6):H2533-2544.
31. Jones DL, Wagers AJ. No place like home: anatomy and function of the stem cell niche. *Nat Rev Mol Cell Biol*. Jan 2008;9(1):11-21.
32. Meirelles Lda S, Nardi NB. Methodology, biology and clinical applications of mesenchymal stem cells. *Front Biosci (Landmark Ed)*. Jan 01 2009;14:4281-4298.
33. Ivanovic Z. Hypoxia or in situ normoxia: The stem cell paradigm. *J Cell Physiol*. May 2009;219(2):271-275.
34. Packer L FK. Low oxygen concentration extends the lifespan of cultured human diploid cell. *Nature*. 1977(267):423-425.
35. De Bari C, Dell'Accio F, Tylzanowski P, Luyten FP. Multipotent mesenchymal stem cells from adult human synovial membrane. *Arthritis Rheum*. Aug 2001;44(8):1928-1942.
36. Covas DT, Siufi JL, Silva AR, Orellana MD. Isolation and culture of umbilical vein mesenchymal stem cells. *Braz J Med Biol Res*. Sep 2003;36(9):1179-1183.
37. Bruder SP, Jaiswal N, Haynesworth SE. Growth kinetics, self-renewal, and the osteogenic potential of purified human mesenchymal stem cells during extensive subcultivation and following cryopreservation. *J Cell Biochem*. Feb 1997;64(2):278-294.
38. Ades EW, Zwerner RK, Acton RT, Balch CM. Isolation and partial characterization of the human homologue of Thy-1. *J Exp Med*. Feb 01 1980;151(2):400-406.
39. Duff SE LC. CD105 is important for angiogenesis: evidence and potential applications. *FASEB J*. 2003;17(9):8.

40. Gougos A, St Jacques S, Greaves A, et al. Identification of distinct epitopes of endoglin, an RGD-containing glycoprotein of endothelial cells, leukemic cells, and syncytiotrophoblasts. *Int Immunol*. Jan 1992;4(1):83-92.
41. Beyer Nardi N, da Silva Meirelles L. Mesenchymal stem cells: isolation, in vitro expansion and characterization. *Handb Exp Pharmacol*. 2006(174):249-282.
42. Trounson A, McDonald C. Stem Cell Therapies in Clinical Trials: Progress and Challenges. *Cell Stem Cell*. Jul 02 2015;17(1):11-22.
43. Trounson A, Thakar RG, Lomax G, Gibbons D. Clinical trials for stem cell therapies. *BMC Med*. May 10 2011;9:52.
44. Reinders ME, Bank JR, Dreyer GJ, et al. Autologous bone marrow derived mesenchymal stromal cell therapy in combination with everolimus to preserve renal structure and function in renal transplant recipients. *J Transl Med*. Dec 10 2014;12:331.
45. Liebergall M SJ. Stem cell-based therapy for prevention of delayed fracture union: a randomized and prospective preliminary study. *Molecular Therapy* 2013;8(21):8.
46. Orozco L, Munar A, Soler R, et al. Treatment of knee osteoarthritis with autologous mesenchymal stem cells: a pilot study. *Transplantation*. Jun 27 2013;95(12):1535-1541.
47. Orozco L, Soler R, Morera C, Alberca M, Sanchez A, Garcia-Sancho J. Intervertebral disc repair by autologous mesenchymal bone marrow cells: a pilot study. *Transplantation*. Oct 15 2011;92(7):822-828.
48. Chang YS, Ahn SY, Yoo HS, et al. Mesenchymal stem cells for bronchopulmonary dysplasia: phase 1 dose-escalation clinical trial. *J Pediatr*. May 2014;164(5):966-972 e966.
49. Li TS, Hamano K, Suzuki K, Ito H, Zempo N, Matsuzaki M. Improved angiogenic potency by implantation of ex vivo hypoxia prestimulated bone marrow cells in rats. *Am J Physiol Heart Circ Physiol*. Aug 2002;283(2):H468-473.
50. Panchision DM. The role of oxygen in regulating neural stem cells in development and disease. *J Cell Physiol*. Sep 2009;220(3):562-568.
51. Bates MK. Culturing cells under hypoxic conditions for biologically relevant results *American laboratory*. 2012;1(2).
52. Kretlow JD, Jin YQ, Liu W, et al. Donor age and cell passage affects differentiation potential of murine bone marrow-derived stem cells. *BMC cell biology*. Oct 28 2008;9:60.
53. Rebelatto CK, Aguiar AM, Moretao MP, et al. Dissimilar differentiation of mesenchymal stem cells from bone marrow, umbilical cord blood, and adipose tissue. *Exp Biol Med (Maywood)*. Jul 2008;233(7):901-913.
54. Annabi B LY, Turcotte S, Naud E. Hypoxia promotes murine bone marrow derived stromal cell migration and tube formation. *Stem Cells*. 2003(21):337-347.
55. Sekiya I LB, Smith JR. Expansion of human adult stem cells from bone marrow stroma: conditions that maximize the yields of early progenitors and evaluate their quality. *Stem Cells*. 2002(20):530-541.
56. Rubio D, Garcia-Castro J, Martin MC, et al. Spontaneous human adult stem cell transformation. *Cancer Res*. Apr 15 2005;65(8):3035-3039.
57. al Ale. The effects of short-term hypoxia on human mesenchymal stem cell proliferation, viability and p16INK4A mRNA expression: Investigation using a simple hypoxic culture system with a deoxidizing agent. *Journal of stem cells and regenerative medicine*. 2015;11(1):6.
58. Cipolleschi MG, Dello Sbarba P, Olivotto M. The role of hypoxia in the maintenance of hematopoietic stem cells. *Blood*. Oct 01 1993;82(7):2031-2037.

59. Tsai CC, Chen YJ, Yew TL, et al. Hypoxia inhibits senescence and maintains mesenchymal stem cell properties through down-regulation of E2A-p21 by HIF-TWIST. *Blood*. Jan 13 2011;117(2):459-469.
60. Braun J HA, Weis-Klemm M. Evaluation of the osteogenic and chondrogenic differentiation capacities of equine adipose tissue-derived mesenchymal stem cells. *Am J Vet Res*. 2010;71(10):1228-1236.
61. Pascucci L CG, Mercati F. Flow cytometric characterization of culture expanded multipotent mesenchymal stromal cells from horse adipose tissue: Towards the definition of minimal stemness criteria *Vet immunol immunopathol*. 2011;144(3-4):499-506.
62. Valorani M. Mesenchymal Stem Cells Grown Under Hypoxic Conditions: Compositions, Methods and Uses Therefor *United states patent application publication*. 2010:11.
63. Van G HJ, Kanaar, . Chromosomal stability and the DNA double-stranded break connection. *Nat Rev Genet*. 2001;2:196-206.
64. Riekstina U, Cakstina I, Parfejevs V, et al. Embryonic stem cell marker expression pattern in human mesenchymal stem cells derived from bone marrow, adipose tissue, heart and dermis. *Stem Cell Rev*. Dec 2009;5(4):378-386.
65. Hypoxia is no hype: Perspectives across Phylogeny, Stem Cell differentiation & Geochemistry. *Journal of stem cells and regenerative medicine*. 2015;11(1):1.
66. Mohyeldin A, Garzon-Muvdi T, Quinones-Hinojosa A. Oxygen in stem cell biology: a critical component of the stem cell niche. *Cell Stem Cell*. Aug 06 2010;7(2):150-161.