Conclusions. The high frequency of chromosomal abnormalities among patients with fertility requires more attention from doctors of reproductive medicine. Peripheral blood cells' karyotyping appears to be one of the most important steps in couple's examination held before their enterance the in vitro fertilization program. This test is crucial for the calculation of risk of chromosomally abnormal child conceiving and subsequent choice of the most appropriate treatment approach involving additional diagnostic methods, e.g. preimplantation genetic diagnosis. This procedure allows to avoid the embryotransfer of aneuploid or chromosomally unbalanced embryos into the uterus what is extremely helpful in case of one of the parents is a carrier of balanced chromosomal aberrations or other abnormality.

References

1. Chantot-Bastaraud S, at al. Underlying karyotype abnormalities in IVF/ICSI patients // Reprod Biomed Online. – 2008. – Vol.16, № 4: – P.514-22.
2. Düzcan F at al. Cytogenetic studies in patients with reproductive failure// Acta Obstet Gynecol Scand. – 2003 – Vol. 82, № 1. – P.53-6.
3. Gekas J at al. Chromosomal factors of infertility in candidate couples for ICSI: an equal risk of constitutional aberrations in women and men // Hum Reprod. – 2001. – Vol. 16, № 1. – P. 82-90.
4. Gekas J at al. Value of karyotyping women patients of couples referred for sterility// Gynecol Obstet Fertil. – 2003. – Vol. 3, № 1. – P: 66-9.
5. Morel F at al. Chromosomal abnormalities in couples undergoing intracytoplasmic sperm injection. A study of 370 couples and review of the literature// Int J Androl. – 2004. – Vol. 27, № 1. – P. 178-82.
6. Peschka B, at al. Type and frequency of chromosome aberrations in 781 couples undergoing intracytoplasmic sperm injection // Hum Reprod. – 1999. – Vol. 14. № 9. – P. 2257-63.
7. Radojcić Badovinac A at al. Chromosome studies in patients with defective reproductive success// Am J Reprod Immunol. – 2000. – Vol. 44, № 5. – P. 279-83.
8. Атраментова Л.А., Утевская О.М. Статистические методы в биологии: Учебник. – Горловка: ЧП "Видавництво Ліхтар", 2008.
9. Ворсанова С.Г., Юров Ю.Б., Чернышов В.Н. – Хромосомные синдромы и аномалии. // Ростов-на Дону – 1999. – с.155-156.
10. Глинкина Ж.И. Комплексное генетическое обследование мужчин: программа ИКСИ// Гинекология, том08/N, М. – 2006. – с. 28-30.
11. Зерова-Любимова Т.Е., Горовенко Н.Г. Стандарты анализа препаратов хромосом человека (методические рекомендации) // К., 2003. – с.10.

Received to editorial board 21.06.13

Ю. Гонтар, асп., О.Чапля, асп., І. Ільїн, д-р мед. наук
КНУ імені Тараса Шевченка, Київ

ПОРУШЕННЯ КАРІОТИПУ СЕРЕД ЧОЛОВІКІВ І ЖІНОК З ДІАГНОЗОМ БЕЗПЛІДДЯ

Дана стаття висвітлює частоту хромосомних аномалій серед жінок і чоловіків з порушенням репродуктивної функції та безпліддям.
Ключові слова: каріотип, безпліддя, цитогенетичні дослідження, транслокації, інверсії, мозаїцизм.

Ю. Гонтарь, асп., О. Чапля, асп., И. Ильин, д-р мед. наук
КНУ имени Тараса Шевченко, Киев

НАРУШЕНИЕ КАРИОТИПА СРЕДИ МУЖЧИН И ЖЕНЩИН С ДИАГНОЗОМ БЕСПЛОДИЕ

Данная статья освещает частоту хромосомных аномалий у женщин и мужчин с нарушениями репродуктивной функции и бесплодием.
Ключевые слова: кариотип, бесплодие, цитогенетические исследования, транслокации, инверсии, мозаицизм.

UDC 619:611.013:57.085.2:636

A. Mazurkevich, Doct. Vet. Sc., L. Kladnytska, PhD., V. Kovpak, PhD
National University of Life and Environmental Sciences of Ukraine, Kyiv

FEATURES CONDITIONS SELECTION AND CULTIVATION MOUSE BONE MARROW ADHEZIVE FRACTION MONONUKLEAR CELLS

Was established that the parameters centrifugation of mouse bone marrow cells in density gradient fikol-verohrafin 1,074 by centrifugal force of 300 g is provide getting an adhesive cells fraction, the most enriched on mesenchymal stem cells. The conditions for the allocation of mouse bone marrow cells, in which a mixture contributes most proliferative activity of mesenchymal stem cells (MSC).

Key words: mesenchimal stem cells, fikol-verohrafin gradient, bone marrow, mouse.

Introduction. For the formation and growth of the colonies of mononuclear bone marrow cells requires some concentration and ratio of substances and factors that stand in the environment in the life of most cells. For a variety of techniques and correlation of these substances varies, which in turn affects the formation and growth of colonies of cells.

It is known that the cells of organism need to exchange information with each other to regulate its development of the tissue, to control growth process and division, to coordinate functions. The structure of the bone marrow are fibroblasts, macrophages, adipocytes, osteoblasts, osteoclasts, endothelial cells, mesenchymal and hematopoietic stem cells and their descendants [3,4].

Vital cells in culture greatly simplified compared to the functioning of the body, and is usually limited to division. Interactions of bone marrow cells supports stay mesenchymal stem cells in the inactive phase of mitosis (G0). The output of this phase of the transition in the mitotic cycle is regulated by changes in intracellular signals [5,1]. Intercellular interaction occurs through hormones, neurotransmitters, and histohormoniv. Obviously, the latter type of intercellular signaling is present between the cells of the bone marrow, and the signals received by the bone marrow cells, and report violations of homeostasis in the body, coming through hormonal and neurotransmitter substances. These signals and give the command to exit stimulates a number of stem cells from dormantnoho state, which then become the path of differentiation and migrate to the area of the pathological process under the action of the same intercellular signals [2,3]. The same signaling molecules can cause target cells unequal response. In some cases this is due to the fact that the signaling molecule binds to various proteins, receptors, in other cases, they bind to the same protein receptors, but activated in different cells different mechanisms of response. Some reaction cells are gradual and increase in direct proportion to the increase ligand. However, there is another group of cells that responds to an increase in the concentration of signaling substances on the principle "all or nothing", in this case, the target cells are not observed any changes until the ligand concentration reaches a threshold level, and the achievement of it – immediately starts maximal response [6].

As already noted, one of the hallmarks of MSCs is their high adhesive ability to culture dishes by culturing. It is

possible, in due time, O.Frydenshteyn separate MSCs from hematopoietic cells. Note that when using this property resultant cell population is heterogeneous and contains other cells that also have adhesive properties and which can, according to some researchers, inhibit proliferative activity receiving MSCs [7, 8].

Materials and methods. The aim of the study was to determine the optimal conditions for isolation and cultivation mouse bone marrow mononuklear cells fraction with high proliferative activity.

We have studied 16 Number of combinations of a cell mononuklernoyi fraction of mouse bone marrow high proliferative activity. It was studied four combinations of method separating suspensions of bone marrow cells in a 4 density gradient fikol-verohrafin (1,074, 1,076, 1,078, 1,080) with the magnitude of the centrifugal force of 300 g and cell culture in vitro on culture medium DMEM, RPMI, 199 with Earle salts, 199 Hepes, with the addition of 20% FBS (fetal calf serum), 10 mkl/sm3 – antibiotic-antifungal.

Cells were seeded in to culture dishes with a density 250 x 10^3 per cm^2. Cultivation of cells was carried out in Petri dishes (d = 60 mm) in the CO_2 incubator with temperature 37 ^oC and CO_2 concentration – 5%. Evaluating the effectiveness of methods for isolation of mononuclear cell fraction of bone marrow was performed based on the formation of colonies and proliferative activity of cells. Evaluation of adhesive properties of cell growth and colony formation, monolayer formation of mononuclear cell fraction of bone marrow was performed every day.

Results and discussion. For various combinations of methods of separating suspensions primary material in a 4 fikol density gradient have unequal number of mononuclear cell fraction of bone marrow. The indicators fikol density gradient and mononuclear cell fraction of bone marrow are directly proportional.

On the 4-5th day of cultivation primary material we have seen the emergence of single cells r sprawled on the bottom of culture dishes and entered the mitotic division (Fig. 1).

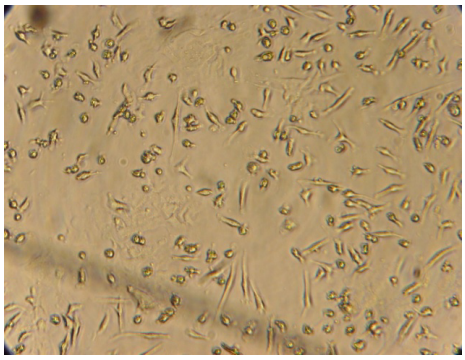

Figure. 1. Mouse MSCs cultured in culture medium DMEM, 4-day cultivation

Violation of genetically rooted combination of bone marrow cells alters cell-cell interactions circuit and causes the change of behavior of cells, which strongly depends on the combination of cells has been flagged for centrifugation in density gradients of different sizes. Over such a mechanism is out of dormantnoho MSC status in primary culture. This may be an explanation of our results with very high deviation between groups of experiments (Table 2).

Revealed that the parameter density gradient centrifugation at 1,074 is most appropriate for a population of cells with the highest proliferative activity, namely the 9 days of culturing the resulting cell population was already formed a continuous monolayer (Fig. 2).

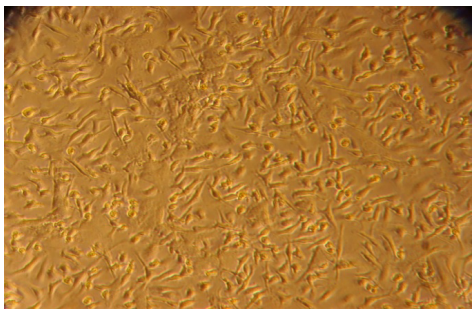

Figure. 2. Mouse MSCs cultured in DMEM culture medium by centrifugation of initial parameter in the fikol density gradient material – 1,074, 9-day cultivation

Cell samples from density gradient 1,076 covered only the surface of the culture dishes by 45 and 60% (Figure 3, Figure 4). After a 9-day cultivation experiments in samples from primary material parameters centrifugation in density gradient fikolu 1,078 and 1,080 received by us forming monolayer cells within 40-50%.

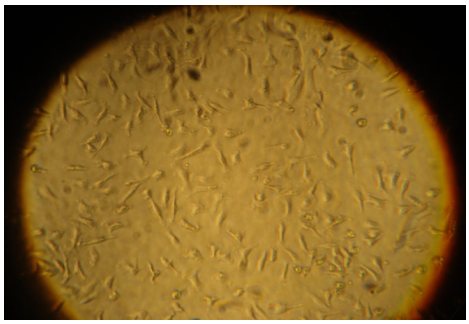

Figure. 3. Mouse MSCs Mouse MSCs cultured in culture medium RPMI, centrifugation parameter for the primary material of fikol gradient density – 1,076, 9-day cultivation

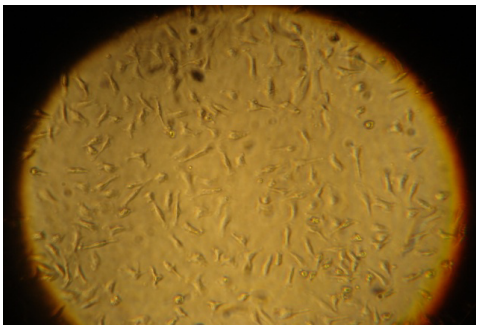

Figure. 4. Mouse MSCs cultured in culture medium DMEM, centrifugation parameter for the primary material of fikol gradient density 1,076, 9-day cultivation

Conclusions. Thus, the parameters centrifugation suspension mouse bone marrow cells in fikol density gradient 1, 074 with centrifugal force of 300 g provide a fraction of cells, the most enriched for MSCs, and a mixture of bone marrow cells obtained by us in these parameters, the most promotes proliferative activity of mouse MSCs. Obviously, under these conditions in the resultant fraction is more MSCs.

References

1. Bianco P. Bone marrow stromal stem cells: nature, biology, and potential applications / P. Bianco, M. Riminucci, S. Gronthos et.al. // Stem cells. – 2001. – № 19. – P.180–192.

2. Кухарчук А.Л. Стволовые клетки: эксперимент, теория, клиника. Эмбриональные, мезенхимальные, нейральные и гемопоетические

стволовые клетки / Кухарчук А.Л., Радченко В.В., Сирман В.М. – Черновцы: Золоті Литоври, 2004. – 505 с.

3. Мушкамбаров Н.Н. Молекулярная биология/ Н.Н. Мушкамбаров, С.Л. Кузнєцов. – М.: Медицинское информационное агенство, 2003. – 535 с.

4. Мушкамбаров Н.Н. Молекулярная биология/ Н.Н. Мушкамбаров, С.Л. Кузнєцов. – М.: Медицинское информационное агенство, 2003. – 535 с.

5. Hart P.H. Mononuclear cell factors, that inhibit fibroblast collagen synthesis. *In vitro* conditions determinity their production and Expression./ P.H. Hart, L.W. Powell, W.G.E. Cooksley [et al.]//Scand. J. Immunol. – 1985. – Vol.18. – P.51–58.

6. Metzyer Z. Suppression of fibroblast proliferation by activated macrophages: Involvment of H_2O_2 and a non-prostaglandin E product of the cyclooxygenase pattway / Z. Metzyer, J.T. Hoffeld, J.J. Oppenheim // Cell. Immmunol. –1986. –Vol. 100. – № 2. – P.501–514.

7. Чупикова Н.И. Получение и биологическая характеристика мультипотентных мезенхимных стромальных клеток костного мозга человека и создание на их основе костных структур: дис. канд. биол. наук : 03.00.23/ Чупикова Наталия Игоревна. – М., 2007. – 133 с.

8. Фриденштейн А.Я. Пролиферативные и дифференцировочные потенции костномозговых колониеобразующих клеток / А.Я. Фриденштейн, Р.К. Чайлахян, Ю.В. Герасимов // Цитология. – 1986. – №28(3). – С.341–349.

Received to editorial board 21.06.13

А. Мазуркевич, Л. Кладницька, В. Ковпак
Національний університет біоресурсів та природокористування України, Київ

ОПТИМАЛЬНІ УМОВИ ВИДІЛЕННЯ ТА КУЛЬТИВУВАННЯ АДГЕЗИВНОЇ ФРАКЦІЇ МОНОНУКЛЕАРНИХ КЛІТИН КІСТКОВОГО МОЗКУ МИШІ

Встановлено, що параметри центрифугування клітин кісткового мозку миші в градієнті щільності фікол-верографіну 1,074, з відцентровою силою 300 g забезпечують отримання адгезивної фракції клітин, найбільш збагаченої на мезенхімальні стовбурові клітини. Визначено умови виділення клітин кісткового мозку миші, за яких їх суміш найбільш сприяє проліферативній активності мезенхімальних стовбурових клітин.

Ключові слова: мезенхімальні стовбурові клітини, фікол-верографін.

А. Мазуркевич, Л. Кладницкая, В. Ковпак
Национальный университет биоресурсов и природопользования Украины, Киев

ОПТИМАЛЬНЫЕ УСЛОВИЯ ВЫДЕЛЕНИЯ И КУЛЬТИВИРОВАНИЯ АДГЕЗИВНОЙ ФРАКЦИИ МОНОНУКЛЕАРНЫХ КЛЕТОК КОСТНОГО МОЗГА МЫШИ

Установлено, что параметры центрифугирования клеток костного мозга мышей в градиенте плотности фикол-верографина 1,074, с отцентровой силой 300 g обеспечивают получение адгезивной фракции клеток, наиболее обогащенной мезенхимальными стволовими клетками. Установлены условия выделения клеток костного мозга мыши, при которых смесь последних наиболее способствует пролиферативной активности мезенхимальных стволовых клеток.

Ключевые слова: мезенхимальные стволовые клетки, фикол-верографин.

UDC 615.373:576.54

A. Bilyuk, student, T. Nikolaenko, student, N. Petruk, student, I. Saraieva, student, O. Dzhus, student, V. Nikulina, PhD stud., O. Andruschenko, student, L. Garmanchuk, Doct. Biol. Sience
Taras Shevchenko National University of Kyiv ESC Institute of biology, Kyiv

EFFECT OF VEGF ON NO-PRODUCTION BY ENDOTHELIAL CELLS

It was established that VEGF ma anti-VEGF have an opposite effect on endothelium cells. VEGF stimulates production of NO and inhibits apoptosis, and anti-VEGD slightly decrease NO production rate and induce apoptosis.

Key words: endothelium cell., apoptosis., VEGF.

Introduction. One of intracellular mediators of angiogenesis is an reactive nitrogen species in particular NO [1]. It was shown that NO has a direct influence on the proliferation and migration of endothelial cells, protein synthesis and increasing of vascular permeability. These are very important processes for the angiogenesis initiation. Upon the blocking of NO synthesis, the proliferation and differentiation of endothelial cells are inhibited. The differention cease appeares by the violation of precapillary structures formation as well as loosing of the endothelial cells ability to migrate in certain direction. It is able both to increase lipid peroxidation in cell membranes and serum lipoproteins. Their inhibition may cause vasodilation, induce apoptosis and show a protective effect against the introduction of other agents [2]. Recent *in vitro* studies have established that the VEGF (vascular endothelial growth factor) stimulates endothelial cells to produce nitric oxide [3]. Vascular endothelial growth factor (VEGF) is a potent endothelial cell-specific mitogen that promotes angiogenesis, vascular hyperpermeability, and vasodilation by autocrine mechanisms involving NO [5]. NO of both endogenous and exogenous origin inhibits apoptosis in a variety of cells and tissues. NO is a small membrane permeable gas that serves as a mediator of many physiological events. It is produced by the oxidation of L-arginine by a family of isoenzymes-NOS (NO-synthase) [4] Today we can surely claim that one of the factors, which stimulates the increasing of NO content in the culture medium, is a factor of endothelial cells (VEGF). It interacts with eNOS in caveolaes in normal endothelial cells, regulates their activity and thereby promotes the production of NO and prostacyclin, as well as through the activation of cytosolic phospholipase A_2 [6]. There is another possible mechanism of VEGF-dependent activation of NO-production connected with involving of the heat shock protein 90. Activation of NO-synthase and increasing of vascular permeability in endothelial cells are instated by interaction of VEGF with the VEGF2 receptor. The feedback was shown. In a certain concentration range NO causes the synthesis of VEGF by HIF-1 (Hypoxia-inducible factor 1)mediated way [7].

Aim. The aim of this study was the comparing of the NO production level in the culture medium of endothelial cells upon the implementation of VEGF and anti-VEGF.

Materials and methods. Mouse endothelial cells (MAEC) [8]., cultivated under standard conditions in DMEM with addition of 10% FBS, were used in this study. In case of long-term cultivation unfed culture model was used, which allows estimating cell's functions under both paracrine and autocrine regulation with intra- and extracellular factors. Effectiveness of angiogenesis was estimated using light microscopy, cytofluorometric analysis and MTT-test. VEGF was used as a specific angiogenesis-inducing factor, while its antagonist – anti-VEGF – as a downregulator.)