NATIONAL AGRICULTURAL AND FOOD CENTRE
RESEARCH INSTITUTE FOR ANIMAL PRODUCTION NITRA

Slovak J. Anim. Sci., 50, 2017 (2): 73–76

ISSN 1337-9984

COMPARISON OF RABBIT ENDOTHELIAL PROGENITOR CELLS AND MESENCHYMAL STEM CELLS: CYTOGENETIC APPROACH

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ABSTRACT

The aim of this preliminary work was to analyze metaphases of endothelial progenitor cells isolated from peripheral blood and mesenchymal stem cells harvested from rabbit bone marrow in order to assess the effect of culture conditions on the karyotype. We analyzed 30 metaphases of both endothelial cells and mesenchymal stem cells of a rabbit. Our preliminary results show that 73.3 % of endothelial cells retained stable number of chromosomes, while in mesenchymal stem cells we recorded moderately lower percentage (66.6 %). Changes in the genome may lead to changes in the gene expression as well as to dysfunctions of the cell activity. Therefore, it is necessary to analyze chromosomal abnormalities in order to monitor culture conditions, particularly in cells intended for the therapeutic use.

Key words: rabbit; endothelial progenitor cells; mesenchymal stem cells; karyotype

INTRODUCTION

Except the endothelial cells that form the vasculature, non-hematologic endothelial progenitor cells (EPCs) are also present in the bloodstream (Asahara *et al.*, 1997; Yin *et al.*, 1997; Shi *et al.*, 1998; Gehling *et al.*, 2000; Lin *et al.*, 2000). Originating from adult bone marrow tissue, EPCs have the similar features as embryonal angioblasts. These precursor cells have the high capacity to proliferate and to differentiate into mature ECs (Hristov and Weber 2003; Urbich and Dimmeler 2004).

Mesenchymal stem cells (MSCs) are present in many adult tissues. The first tissue where the MSCs were identified was bone marrow (BM-MSCs). Stem cells with this kind of origin possess various advantages in comparison to MSCs of other tissue derivates: high osteogenic differentiation capacity, well investigated properties already applied in use with biomaterials, not ethically controversial background (Kang *et al.*, 2012). MSCs are also capable of high proliferation and multi-lineage differentiation (Jin *et al.*, 2013). However, invasivity of the BM-MSCs harvesting initiates the interest in finding more accessible sources of MSCs (Pontikoglou *et al.*, 2011).

In opposite to the embryonic stem cells, in which chromosomal disorder has been widely reported, MSCs are characterized as genetically stable during culture (Borgonovo *et al.*, 2014). However, karyotype changes in both endothelial and mesenchymal stem cells are associated with donor age and their incidence increases after the fifth passage. Previous research has shown that abnormalities in chromosomal count may result in carcinogenesis (Campos *et al.*, 2009; Miyai *et al.*, 2008). Due to tumorogenic threats it is recommended to investigate the chromosomal count after *in vitro* culture. For the purpose of monitoring of chromosomal stability, Moralli *et al.* (2011) suggested the use of microarray-based techniques: Comparative Genomic Hybridization (CGH), single nucleotide polymorphism (SNP) analysis and transcriptional profiling. Nevertheless, from the perspective of clinical routine, the classical cytogenetic protocols, such as G-banding, are the less cost-consuming but sufficient for objective analysis. Therefore, our study

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Received: June 16, 2017
Accepted: July 3, 2017

aimed to analyze metaphases of the endothelial progenitor cells and stem cells isolated from rabbit bone marrow in order to assess the effect of culture conditions on the cell karyotype.

MATERIAL AND METHODS

Biological material

Clinically healthy rabbits of New Zealand White line were used in the experiment. Rabbits were cage held in a partially air-conditioned hall of a rabbit farm at the NPPC Research Institute for Animal Production Nitra, Slovakia. Rabbits were humanely sacrificed and peripheral blood and femoral bones were processed afterwards.

Isolation of endothelial cells

Peripheral blood was collected into the prepared tubes with anticoagulant (EDTA). Rabbit mononuclear cells of the peripheral blood (PBMCs) were isolated using Biocoll (Biochrom, Berlin, Germany) density-gradient centrifugation. Thereafter, cells were resuspended in an EBM-2 basal medium (Lonza, MD, USA) supplemented with recombinant growth factors (kit; EGM-2 SingleQuots™; CC-4176, Lonza), 20 % of fetal bovine serum (FBS) and antibiotics (1 % of penicillin and streptomycin) (Life Technologies). Cells were immediately placed onto T75 tissue culture flasks and cultured until passage 3 (P3), as described by Vašíček *et al*. (2016a).

Isolation of mesenchymal stem cells

Mononuclear cells were isolated using Biocoll solution from the rabbit bone marrow. Centrifuged cells were resuspended in a MEM-Alpha medium supplemented with FBS and antibiotics and then plated into T75 tissue culture flasks. Cells were cultured for 3 weeks until the P3, as previously described by Vašíček *et al*. (2016b).

Karyotype analysis

Samples for chromosome analysis were processed as follows. After passaging, actively growing cells from P3 were incubated with KaryoMAX® Colcemid™ solution in HBSS (Hanks' Balanced Salt Solution,) (Life Technologies, Slovak Republic) for 16 h at 37 °C and 5 % CO_2. Treated cells were washed with Dulbecco's phosphate buffered saline (DPBS; Gibco BRL, USA) and dissociated by 0.05 % trypsin (ThermoFisher, Slovak Republic). After centrifugation (200 g for 10 min), cells were resuspended and incubated in a hypotonic solution (75 mM KCl) for 20 min at 37 °C. Following centrifugation, harvested cells were incubated with 5 ml of chilled fixative consisting of methanol and acetic acid (3:1; v/v) for 10 minutes. Chromosome spreads were prepared by dropping the cell suspension onto pre-chilled glass slides. The air-dried cell spreads were stained by 2 % Giemsa solution (Gibco BRL, USA) and observed under a light microscope at 400 x magnification. A normal rabbit karyotype consists of 22 pairs (2n = 44) of chromosomes. Chromosomal abnormality was defined as following: hyperploidy - > 44, hypoploidy - < 44. The final percentage of abnormal karyotype was defined as (< 2n) + (> 2n).

RESULTS AND DISCUSSION

The samples were divided into two groups according to the cell type. Metaphase of BM-MSCs is shown in Figure 1 and of EPC in Figure 2.

Our preliminary results show that for endothelial cells the percentage of diploid cells was 73.3 %. An abnormal karyotype occurred in 26.6 %, of which 10 % were hyperploid and 16.6 % hypoploid cells. Miyai *et al.* (2008), who harvested corneal endothelial cells from human cadavers, reported that increased aneuploidy occurs not only in older donors but also in later cultures and passages. Our results correspond to this hypothesis of incidence of chromosomal abnormalities along with increased number of passages.

Concerning MSCs, the proportion of diploid cells was lower (66.6 %) in comparison to EPCs, however chi-square test showed no significant differences between EPCs and MSCs. Abnormal karyotype was observed in 33.5 % of cells, of which 10 % were hyperploid and 23.5 % hypoploid. This phenomenon could be due to the fact that MSCs grew slower than EPCs during culture.

Table 1: Karyotype analysis of endothelial progenitor cells and mesenchymal stem cells from bone marrow

Type of cells	Total number of cells (N)	2n % / N	< 2n % / N	> 2n % / N	Total % of abnormal karyotypes
Endothelial progenitor cells	30	73.3 / 22	16.6 / 5	10 / 3	26.6
Mesenchymal stem cells	30	66.6 / 20	23.5 / 7	10 / 3	33.5

N = number of cells; 2n = diploidy (normal); < 2n = aneuploidy: hypoloidy; > 2n = aneuploidy: hyperploidy

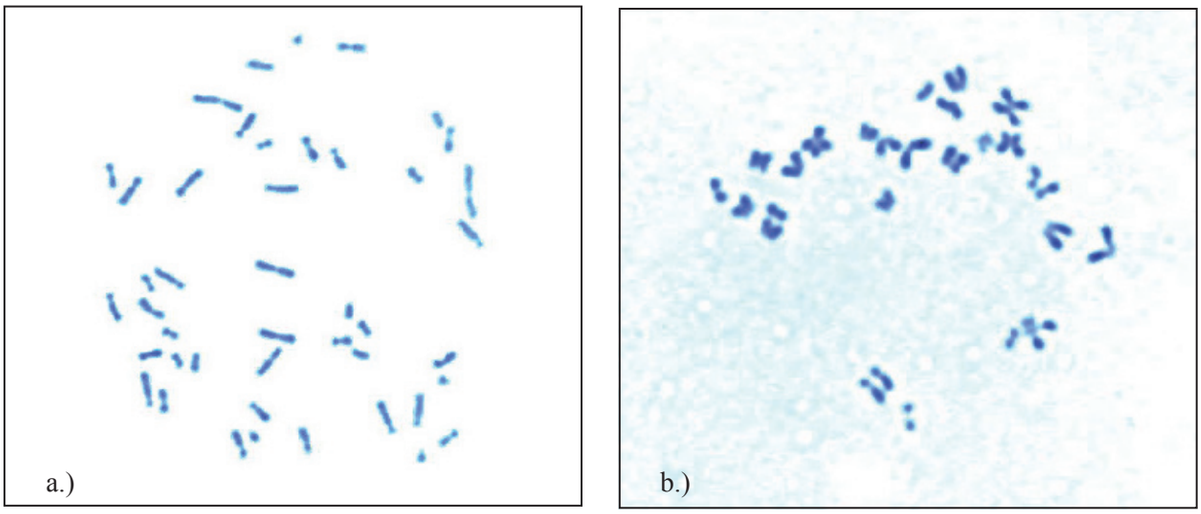

Fig. 1: Bone marrow-derived mesenchymal stem cells: a, normal number b, decreased number – hypoploidy

This outcome does not corresponds with the study of Asadi-Yousefabad *et al.* (2015), who claimed that growing passaging of canine BM-MSCs does not affect the karyotype.

These preliminary findings show that chromosome malformations arise during culture. Observation of karyotypes from the first passage until at least the fifth passage could provide more precise results. Nevertheless, chromosomal aberrations in cells intended for future therapeutic use are a real concern due to association with carcinogenesis (Campos et al., 2009). Therefore, it is recommended to thoroughly evaluate the karyotypes of the cell colonies before differentiation or clinical application.

CONCLUSION

Our preliminary results confirm the importance of cytogenetic study, since this technique is able to detect numeric chromosomal aberrations. Chromosomal abnormalities were detected in both rabbit endothelial progenitor cells and mesenchymal stem cells derived from the bone marrow.

ACKNOWLEDGMENTS

This study was funded by the grant APVV-14-0043 and APVV-14-0348, coordinated by the Slovak Research and Development Agency and VEGA 1/0611/15.

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