Slovak J. Anim. Sci., 40, 2007 (1): 5 - 8

LEVEL OF CHROMOSOMAL ANEUPLOIDY DETECTED FROM PERIPHERAL BLOOD AND BONE MARROW IN TRANSGENIC RABBITS

J. ČURLEJ[1,2], S. ROYCHOUDHURY[2], R. OMELKA[3], P. CHRENEK[1,3]

[1]Slovak Agricultural Research Centre, Nitra, Slovak Republic; [2]Slovak University of Agriculture in Nitra, Slovak Republic; [3]Constantine the Philosopher University in Nitra, Slovak Republic

ABSTRACT

Our study aimed at comparing the chromosomal aneuploidy rate between transgenic and non-transgenic rabbits with respect to the source of cells: bone marrow vs. Peripheral blood culture. Samples were obtained from New Zealand White transgenic (carrying human factor VIII gene) and non-transgenic rabbits. C-metaphase plates were evaluated from bone marrow lymphocytes and peripheral blood lymphocyte samples synchronized by the addition of 0.25 μg/ml colcemide. No significant difference in the chromosomal aneuploidy between transgenic (34.45% from blood vs. 62.8% from bone marrow) and non-transgenic (37.78% from blood vs. 56.1% from bone marrow) rabbits was observed. Higher chromosomal aneuploidy rate was detected from samples of bone marrow than from the peripheral blood cells. In conclusion: hFVIII transgene does not influence aneuploidy rate in transgenic rabbits.

Key words: transgene, rabbit, hFVIII, bone marrow lymphocytes, peripheral blood lymphocytes, chromosomal aneuploidy.

INTRODUCTION

Transgenic rabbits have become a useful model in the study of mechanisms of human diseases such as Parkinson's disease, multiple sclerosis, artheriosclerosis, hemophilia and others. They are also used as a model organism in therapeutic protein production in mammary gland.

Transgenesis, as a process modifiing animal genome could negatively affect a genome of transgene carriers. Karyotype evaluation is useful to reveal possible changes on chromosomal level as are numerical chromosomal changes: increase of aneuploidy or polyploidy and structural chromosomal aberrations: deletions, inversions, translocations and others.

Aneuploidy involves a numerical changes in chromosomal sets and is mostly caused by the loss of chromosomes. The main reason of this is non-disjunction event due to mitotic cell cycle, when mother cell homologous chromosome pairs are incorrectly divided between two daughter cells during segregation process. Normal organisms are characterised by the presence of aneuploid cells at low frequency. Diseases or genetic manipulations might cause an increase in the occurence of aneuploidy and higher level of aneuploidy could, in turn, result in genetic diseases or even become lethal to the organism. Cytogenetic analysis of transgenic or non-transgenic animals may provide with selection markers for the breeding process of these animals.

Our study aimed at comparing the chromosomal aneuploidy rate between transgenic and non-transgenic rabbits with respect to the source of cells: bone marrow vs. peripheral blood culture.

MATERIAL AND METHODS

Biological material

Transgenic (mWAP-hFVIII gene construct, Chrenek et al., 2005) and non-transgenic rabbit siblings were produced by breeding transgenic female with non-transgenic male. Transgenic and non-transgenic rabbits of about 9 months old were analyzed. The animals were housed in individual flat – deck wire cages, under a

Correspondence: E-mail: jojonr@zoznam.sk

constant photoperiod of 14 h of day-light. The temperature and humidity of the building were recorded continuously by means of a thermograph positioned at the same level as the cages. The rabbits were fed *ad libitum* with a commercial diet and water was provided *ad libitum* with nipple drinkers. The breeding conditions were similar to the intensive industrial ones.

Chromosome preparation

Peripheral blood culture

Blood samples at 1ml were isolated using sterile needle and sterile syringe with the anticoagulation substance (Heparin) from rabbit ear vein. In aseptic (under laminar hood) conditions 6 drops of isolated blood were added into 4ml of cultivation medium PB Max Karyotyping (Gibco BRL). Laboratory tubes with culture were cultivated at 37°C in thermobox for 72 hours. For cell cycle synchronization 0.25 µg/ml (Gibco BRL) of colcemide was added to blood culture for 1 hour continuous cultivation under the same thermobox conditions. Cells were hypotonized in 0.075M KCl hypotonic solution for 20 minutes in thermobox. Fixation was done by addition of fresh and cooled (4°C) fixator (3:1 methylalcohol : acetic acid), the treatment was repeated at least three times. Finally, two or three drops of cell suspension were dropped on slides. Dried samples were stained with 2% Giemsa-Romanovski solution for 7 minutes for microscopic observation.

Bone marrow

Samples of bone marrow cells for cytogenetic analysis were taken from transgenic and non-transgenic rabbits, as described by Parkanyi (1981). Briefly, bone marrow cells were flushed from diaphysis of femur by TCM 199 medium (Gibco BRL). Cells were resuspended and incubated in TCM 199 + 0.25 µg/ml colcemide (Gibco BRL) at 37°C for 60 min. After the incubation, the cells were subjected to 20 min treatment with hypotonic solution of potassium chloride (0.075M) followed by fixations in modified Carnoy's solution (3:1 methanol : acetic acid). Resuspended cells were then spread on frozen glass microslides,air-dried, and stained for 7 min with 2% Giemsa solution. Stained microslides were inspected under the Leica microscope. Chromosomal analysis was carried out from chromosome microphotographs, using 30 C-metaphases for each rabbit.

Statistics

The χ^2 test was used to compare the chromosomal aneuploidy of bone marrow and peripheral blood cells in transgenic and non-transgenic rabbits.

RESULTS

In this study we focused on chromosomal aneuploidy evaluation from the peripheral blood lymphocytes and bone marrow lymphocytes of transgenic

Table 1: Distribution of c-metaphases of rabbit peripheral blood cells according to ploidy

Rabbit	Diploidy (2n=44)	Polyploidy (4n)	Hypodiploidy (2n<44)	Aneuploidy Hyperdiploidy (2n>44)	Σ
Transgenic					
♂ T1	22	1	5	2	7
♀ T2	17	1	10	2	12
♂ T3	18	1	8	3	11
♂ T4	16	2	10	2	12
♂ T5	19	2	7	2	9
♂ T6	17	2	10	1	11
Average:	18,17 (60,56%)	1,5 (5%)	8,33 (27,78%)	2 (6,67%)	10,33 (34,45%)
Non-transgenic					
♀ N1	17	0	11	2	13
♀ N2	19	1	7	3	10
♀ N3	15	2	13	0	13
♂ N4	16	2	10	2	12
♀ N5	18	1	9	2	11
♀ N6	20	1	8	1	9
Average:	17,5 (58,33%)	1 (1,17%)	9,67 (32,22%)	1,67 (5,56%)	11,33 (37,78%)

Table 2: Distribution of c-metaphases of rabbit bone marrows according to ploidy

Rabbit	Diploidy (2n=44)	Polyploidy (4n)	Hypodiploidy (2n<44)	Aneuploidy Hyperdiploidy (2n>44)	Σ
Transgenic					
♂ T1	9	1	15	5	20
♀ T2	9	0	15	6	21
♂ T3	8	3	18	1	19
♂ T4	11	3	14	2	18
♂ T5	12	2	7	9	16
♂ T6	11	0	16	3	19
Average:	10 (33,34%)	1,5 (5%)	14,2 (47,22%)	4,33 (14,45%)	18,83 (62,8%)
Non-transgenic					
♀ N1	12	2	13	3	16
♀ N2	10	1	16	3	19
♀ N3	13	2	11	4	15
♂ N4	11	2	15	2	17
♀ N5	12	0	14	4	18
♀ N6	12	2	14	2	16
Average:	11,67 (38, 9%)	1,5 (5%)	13,83 (46,1%)	3 (10%)	16,83 (56,1%)

and non-transgenic rabbits. No significant difference in chromosomal aneuploidy rate between transgenic (34.45% and 62.8%) and non-transgenic (37.78% vs. 56.10%) rabbits was observed for both the peripheral blood and bone marrow, respectively. Most of the aneuploidy was attributed to the category of hypodiploid cells, whereas the frequency of hyperdiploid cells was significantly lower (Tab. 1, 2). Transgenic rabbits exhibited lower numbers of diploid somatic cells than non-transgenic ones. Polyploid cells occured sporadically.

DISCUSSION

Karyotype instability can be a consequence of genetic manipulations due to transfer of a foreign gene. A low level of abnormal chromosomal cells is not thought to be detrimental, because these cells can be eliminated in early development or diverted to extraembryonic structures (Mahmoud et al., 2002). As it was reported, chromosomal aneuploidy can result from meiotic and mitotic non-disjuncion events (Goepfert et al., 2000, Shi et al., 2004) or it may arise as a result of genetic manipulations. Parkanyi et al. (2004) reported that the level of aneuploidy increases with age. Hughes (1968) found in his experiment with Syrian hamsters of different ages, that aneuploidy was present only in 16 months old hamsters.

In our experiment, higher rate of chromosomal aneuploidy was found in bone marrow compared to peripheral blood. Most of aneuploidy was attributed by hypodiploid cells, the frequency of hyperdiploid cells was lower, as shown in the study carried out by Parkanyi et al. (2004). Polyploid cells occured in low percentage. The rate of aneuploidy in different studies also varied depending on the cell source. Austin (1967) reported 63% frequency of aneuploidy in rabbit blastocysts, whilst Shi et al. (2004) observed 83% aneuploidy in rabbit embryos derived from nuclear transfered cumulus cells and 56% in *in vitro* fertilized ones. Parkanyi et al. (2004) and Zartman & Fechheimer (1967) reported lower aneuploidy (35% and 17% respectively) from bone marrow cells of non-transgenic rabbits in contrast to our results. Significantly higher chromosomal aneuploidy rate was detected by Parkanyi et al. (2004) from c-metaphase spreads of peripheral blood lymphocytes of F1 generation transgenic rabbits (60%) as compared to non-transgenic ones (35%), however, without any negative effect on their health or reproduction. Aneuploidy from peripheral blood in our work differs from the above mentioned results for transgenic rabbits and is similar for non-transgenic ones.

In conclusion, higher chromosomal aneuploidy rates were detected from bone marrows than from peripheral blood cells. This can be explained based on the fact that bone marrows, as a source of blood cells is under constant rapid process of development and hence are more prone to mistakes in mitotic cell cycle than the peripheral blood.

ACKNOWLEDGEMENTS

This work was supported by the grant provided by Ministry of Agriculture, Slovak Republic No.: E12.

REFERENCES

AUSTIN, C. R. 1967. Biological mechanisms in aging. In: *Nature*, vol. 213, 1967, p. 1018-1019.

GOEPFERT, T. M. – McCARTHY, M. – KITTRELL, F. S. – STEPHENS, C. – ULLRICH, R. L. – BRINKLEY, B. R. - MEDINA D. 2000. Progesterone facilitates chromosome instability (aneuploidy) in p53 null normal mammary epithelial cells. In: *FASEB J*., vol. 14, 2000, p. 2221-2229.

HUGHES, D. T. 1968. Cytogenetical polymorphism and evolution in mammalian somatic cell populations in vivo and in vitro. In: *Nature*, vol. 217, 1968, p. 518-523.

CHRENEK, P. – VAŠÍČEK, D. – MAKAREVICH, A.V. – JURČÍK, R. – SÜVEGOVÁ, K. – BAUER, M. – PARKÁNYI, V. – RAFAY, J. – BÁTOROVÁ, A. – PALEYANDA, R. K. 2005. Increased transgene integration efficiency upon microinjection of DNA into both pronuclei of rabbit embryos. In: *Transgenic Res*., vol. 14, 2005, p. 417-428.

MAHMOUD, K.Gh.M. – HAMAM, A.M. – NAWITO, M. F. – SEIDA, A.A.M. – NAWAR, S.M.A. 2002. Cytogenetic analysis of early stage buffalo embryos matured and fertilized in vitro. In: *Egypt. J. Vet. Sci*., vol. 36, 2002, p. 87-98.

PARKÁNYI, V. – CHRENEK, P. – RAFAY, J. – SÜVEGOVÁ, K. – JURČÍK, R. – MAKAREVICH, A.V. – PIVKO, J. – HETÉNYI, L. – PALEYANDA, R. K. 2004. Aneuploidy in the transgenic rabbit. In: *Folia Biologica* (Praha), vol. 50, 2004, p. 194-199.

PARKÁNYI, V. 1981. Utilization of some methods of cytogenetic analysis in the detection of chromosome anomalies in the species Oryctolagus cuniculus. In: *Agriculture*, vol. 27, 1981, p. 148-154.

SHI, W. – DIRIM, F. – WOLF, E. – ZAKHARTCHENKO, V. – HAAF, T. 2004. Methylation reprogramming and chromosomal aneuploidy in *in vitro*-fertilized and cloned rabbit preimplantation embryos. In: *Biol. Reprod*., vol. 71, 2004, p. 340-347.

ZARTMAN, D. L. – FECHHEIMER, N. S. 1967. Somatic aneuploidy and polyploidy in inbred and linecross cattle. In: *J. Anim. Sci*., vol. 26, 1967, p. 252-258.

Authors´ address: Jozef Čurlej, Peter Chrenek, Slovak Agricultural Research Centre, Hlohovska 2, 949 92 Nitra, Slovak Republic, fax: 037 65 46 189, jojonr@zoznam.sk, chrenekp@yahoo.com;
Jozef Čurlej, Shubhadeep Roychoudhury, Department of Animal Physiology, Faculty of Biotechnology and Food Sciences, Slovak University of Agriculture in Nitra, Tr. A. Hlinku 2, 949 70 Nitra, Slovak Republic, shubhadeepr@rediffmail.com;
Radoslav Omelka, Peter Chrenek, Faculty of Natural Sciences, Department of Botany and Genetics, Constantine the Philosopher University in Nitra, Tr. A. Hlinku 1, 949 70 Nitra, Slovak Republic, romelka@ukf.sk.