Folia biologica (Kraków), vol. **55** (2007), No 3-4

Short Note

The Effect of hFVIII Transgene on the Chromosomal Aneuploidy Rate in Rabbits

Jozef CURLEJ, Vladimir PARKANYI, Jozef BULLA, Rastislav JURCIK, and Peter CHRENEK

Accepted April 16, 2007

CURLEJ J., PARKANYI V., BULLA J., JURCIK R., CHRENEK P. 2007. The effect of hFVIII transgene on the chromosomal aneuploidy rate in rabbits. Folia biol. (Kraków) **55**: 161-164.

The aim of this study was to compare the chromosomal aneuploidy rate between transgenic and non-transgenic rabbits derived from the F4 generation. Chromosomal analysis was carried out on bone marrow samples of New Zealand White transgenic (carrying human factor VIII gene) and non-transgenic rabbits (F4 generation) each having a different genetic background (female no. 1-3-5 line I and female no.1-9-7 line II). C-metaphase plates were obtained from the bone marrow lymphocytes synchronized by the addition of 0.25 μg/ml colcemide. No significant difference in chromosomal aneuploidy between transgenic (61%) and non-transgenic (51.27%) rabbits of line I was observed. A higher but non-significant aneuploidy rate between transgenic and non-transgenic rabbits was found in line II, on the other hand a significant difference ($P<0.05$) was observed in diploidy rate. In conclusion, chromosomal aneuploidy rates in this experiment were higher than published previously in other reports.

Key words: Transgene, rabbit, hFVIII, bone marrow lymphocytes, chromosomal aneuploidy.

Jozef CURLEJ, Slovak Agricultural Research Centre, Hlohovska 2, 949 92 Nitra, Slovak Republic and Faculty of Biotechnology and Food Sciences, Slovak University of Agriculture in Nitra, Slovak Republic.
Vladimir PARKANYI, Rastislav JURCIK, Slovak Agricultural Research Centre, Hlohovska 2, 949 92 Nitra, Slovak Republic.
Jozef BULLA, Faculty of Biotechnology and Food Sciences, Slovak University of Agriculture in Nitra, Slovak Republic.
Peter CHRENEK, Slovak Agricultural Research Centre in Nitra, Slovak Republic and Faculty of Natural Science, Constantine the Philosopher University, Nitra, Slovak Republic.
E-mail: chrenekp@yahoo.com

Transgenic rabbits have become a useful model organism for genomic analyses, the study of gene expression and regulation of recombinant human proteins and enzymes (FAN & WATANABE 2003). Random integration of a transgene after pronuclear microinjection can disrupt the function or regulation of an endogenous gene, resulting in chromosomal aneuploidy (GOEPFERT *et al*. 2000). PARKANYI *et al.* (2004) suggest that the level of aneuploid cells in mammals is genetically determined.

In rabbits, the rate of aneuploidy depends on the origin of the tissue ranging from 5% in *in vitro* fertilized oocytes (ASAKAWA *et al.* 1988), 16-18% in blood cells (PARKANYI 1981), 35% in bone marrow (PARKANYI 1981), up to 56-83% in IVF and cloned embryos (SHI *et al.* 2004). PARKANYI *et al.* (2004) analysed transgenic rabbits with integrated hFVIII gene and observed a significantly higher rate of aneuploidy in c-metaphase spreads of peripheral blood lymphocytes in transgenic individuals (62%) as compared to non-transgenic ones (33%). Chromosomal instability was also shown for bone marrow cells derived from transgenic rabbits from F2 and F3 generations (MARTINIAKOVA *et al*. 2005). As previously pointed out, studies on transgenic animals should also focus on the evaluation of chromosomal aneuplody, which may have serious consequences on health, fertility or yield in trangenic animals.

The objective of this study was to compare the chromosomal aneuploidy rate of transgenic and non-transgenic rabbits derived from the F4 generation with the same genetic background.

Material and Methods

Biological material

Two lines of transgenic (mWAP-hFVIII gene construct, CHRENEK *et al*. 2005) and non-transgenic

offspring (F4 generation), each from the same litters, were produced by breeding transgenic females no.1-3-5 with a non-transgenic male (line I) and transgenic females no. 1-9-7 with a transgenic male (line II). Altogether 5 transgenic and 3 non-transgenic rabbits from line I and 5 transgenic and 2 non-transgenic rabbits from line II, each about 9 months old, were analysed.

The animals were housed in individual flat – deck wire cages, under a constant photoperiod of 14 h of day-light. The temperature and humidity of the building were recorded continually 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 intensive industrial conditions.

Detection of transgene

Total DNA was isolated from the ear tissue of newborn rabbits. Conditions for PCR amplification of the hFVIII transgene were the same as reported by CHRENEK *et al*. (2005), using primers hFVIII-F: 5'-GTA GAC AGC TGT CCA GAG GAA-3' and hFVIII-R: 5'-GAT CTG ATT TAG TTG GCC CAT C-3', which define a 578 bp region of human FVIII cDNA.

Chromosome preparation

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 (Gibco BRL). Cells were resuspended and incubated in TCM 199 + 0.25 μg/ml colcemid (Life Technologies) at 37°C for 40 min. After the incultivation, the cells were subjected to 45-50 min hypotonic treatment with potassium chloride (0.075 M) and fixations in modified Carnoy´s solution (3:1, methanol: acetic acid). Resuspended cells were then spread on frozen glass microslides, air-dried, and stored unstained at room temperature, or stained for 10 min with a 2% Giemsa solution. Stained microslides were observed under a Leica microscope. The chromosomal analysis was carried out from chromosome microphotographs, using 30 C-metaphases for each rabbit according to the international standard for rabbit (Committee for the Standardized Karyotyping of the Domestic Rabbit, 1981).

Statistics

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

Results

In this study we focused on chromosomal aneuploidy evaluated from the bone marrow lymphocyte cells. No significant differences in chromosomal aneuploidy rate between transgenic (61 and 70%) and non-transgenic (51.27 and 61.4%) rabbits either from line I or line II were observed. A significant difference ($P<0.05$) in chromosomal diploidy between transgenic and non-transgenic rabbits of line II was found (Table 2). Most of the aneuploidy was attributable to the category of hypodiploid cells, whereas the frequency of hyperdiploid cells was significantly lower (Table 1, 2). Transgenic rabbits exhibited lower numbers of

Table 1

Distribution of c-metaphases of rabbit bone marrow according to ploidy (female no. 1-3-5)

Rabbit	Diploidy (2n=44)	Polyploidy (4n)	Aneuploidy		
			Hypodiploidy (2n<44)	Hyperdiploidy (2n>44)	Σ
Transgenic					
♀ 9n	10(33%)	1(3%)	14(47%)	5(17%)	19(64%)
♂ 11n	10(33%)	0(0%)	14(47%)	6(20%)	20(67%)
♂ 12n	10(33%)	2(7%)	17(57%)	1(3%)	18(60%)
♀ 16n	10(33%)	2(7%)	15(50%)	3(10%)	18(60%)
♀ 17n	13(43%)	1(3%)	7(23%)	9(30%)	16(53%)
Average:	11(35%)	1(4%)	13(45%)	5(16%)	18(61%)
Non-transgenic					
♀ n	13(43.33%)	1(3.33%)	13(43.33%)	3(10%)	16(53.33%)
♀ 2n	12(40%)	2(7%)	12(40%)	4(13%)	16(53%)
♀ 4n	12(40%)	3(10%)	14(47%)	1(3%)	15(50%)
♀ 5n	13(43.33%)	2(7%)	12(40%)	3(10%)	15 (50%)
♂ 7n	12(40%)	2(7%)	11(37%)	4(13%)	15(50%)
Average:	12.4(41.33%)	2(6.87%)	12.4(41.47%)	3(9.8%)	15.4(51.27%)

Table 2

Distribution of c-metaphases of rabbit bone marrow according to ploidy (female no. 1-9-7)

Rabbit	Diploidy (2n=44)	Polyploidy (4n)	Aneuploidy		
			Hypodiploidy (2n<44)	Hyperdiploidy (2n>44)	Σ
Transgenic					
♀ 10c	7(23%)	0(0%)	15(50%)	8(27%)	23(77%)
♀ 12c	7(23%)	0(0%)	15(50%)	8(27%)	23(77%)
♀ 13c	7(23%)	0(0%)	14(47%)	9(30%)	23(77%)
♀ 14c	7(23%)	5(17%)	12(40%)	5(17%)	17(57%)
♂ 15c	8(27%)	2(7%)	14(47%)	6(20%)	18(60%)
Average:	7(24%)[a]	1(5%)	14(47%)	7(24%)	21(70%)
Non-transgenic					
♀ 2c	11(36.67%)	1(3%)	15(50%)	3(10%)	18(60%)
♀ 3c	10(33.33%)	0(0%)	17(57%)	3(10%)	20(67%)
♀ 7c	12(40%)	0(0%)	17(57%)	1(3%)	18(60%)
♀ 9c	11(37%)	1(3%)	16(53%)	2(7%)	18(60%)
♂ 11c	11(37%)	1(3%)	17(57%)	1(3%)	18(60%)
Average:	11(36.8%)[b]	0.6(1.8%)	16.4(54.8%)	2(6.6%)	18.4(61.4%)

[a]vs[b] significant difference at $P<0.05$

diploid somatic cells than non-transgenic rabbits. Polyploid cells occured sporadically in both groups of rabbits.

Discussion

As reported previously, chromosomal aneuploidy can result from meiotic and mitotic non-disjunction events (GOEPFERT *et al*. 2000, SHI *et al*. 2004) or it may come from genetic manipulations. PARKANYI *et al*. (2004) reported that the level of aneuploidy increases with animal age. A low level of chromosomally abnormal cells is not thought to be detrimental, because these cells can be eliminated in early development or diverted to extraembryonic structures (WARD *et al*. 1993). In this experiment transgenic rabbits were characterized by a higher frequency of aneuploidy depending on line as compared to non-transgenic ones. A relatively higher rate of numerical aneuploidy in our transgenic rabbits from line II compared to the transgenic ones from line I may be explained by their differential genetic background. Transgenic offspring from line II were derived from a transgenic female bred with a transgenic male. In line I the male used for breeding was non-transgenic. Most of the aneuploidy was caused by hypodiploid cells, the frequency of hyperdiploid cells was lower, as shown in the study carried out by PARKANYI *et al*. (2004). On the other hand, non-transgenic rabbits had a higher rate of diploid somatic cells. Polyploid cells occured sporadically in both groups of rabbits. The rate of aneuploidy in different studies also varied depending on the cell source. Significant differences in the numbers of aneuploid and diploid cells between transgenic (70 to 80%) and non-transgenic rabbits (40-56%) from F2 and F3 generations were previously shown, but without deleterious effects on health or reproduction (MARTINIAKOVA *et al*. 2005). PARKANYI *et al*. (1981) reported lower aneuploidy (35%) from bone marrow cells of non-transgenic rabbits in comparison to our results. A significantly high numerical chromosomal aneuploidy rate was detected by PARKANYI *et al*. (2004) from c-metaphase spreads of peripheral blood lymphocyte of transgenic rabbits (about 60%) of F1 generation as compared to non-transgenic ones (about 35%) without any negative effect on their health or reproduction. The higher rate of aneuploidy recorded by in this study in transgenic and non-transgenic rabbits may be explained by the different transgene integration in the genomes of two mothers (female no. 1-3-5, line I) as one copy and more copies (female no. 1-9-7, CHRENEK *et al*. 2006). We suggest that a higher level of aneuploidy was caused by the older age of slaughtered animals (8-9 months), reported also by PARKANYI *et al.* (2004). Other possible factors such as defective centromeres, abnormal centrosomes or the loss of mitotic checkpoints (GOEPFERT *et al*. 2000) may have a serious effect on the aneuploidy level. It is also known that older animals exhibit higher chromsomal aneuploidy, which may be explained by lower cell osmoresistence leading to the elimination of chromosomes during the process of chromosome sample preparation.

High chromosomal aneuploidy was also shown in the rabbit embryo. AUSTIN (1967) reported a 63% frequency of aneuploidy detected in rabbit blastocysts. SHI *et al*. (2004) found 83% aneuploidy in rabbit embryos derived from cumulus cell nuclear transfer and 56% in *in vitro* fertilized ones. KARP and SMITH (1975) reported that 46.4% of oocytes cooled to 23°C had abnormal numbers of chromosomes. These confirm the clear influence of temperature on *in vitro* cultured oocytes which may have an adverse effect on newborn individuals.

In conclusion, these results confirm higher chromosomal aneuploidy in transgenic rabbits from F4 generation compared with previous reports from F1, F2 and F3 generations.

Acknowledgements

The authors are grateful to Alexander MAKAREVICH and Shubhadeep ROYCHOUDHURY for English correction. This study was supported from a grant from the Ministry of Agriculture, Slovak Republic.

References

ASAKAWA T., ISHIKAWA M., SHIMITZU T., DUKELOW W. R. 1988. The chromosomal normality of *in vitro*-fertilized rabbit oocytes. Biol. Reprod. **38**: 292-295.

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

CHRENEK P., RYBAN L., VETR H., MAKAREVICH A., KIRCHEROVA. K., BINDER B. 2006. Rabbit transgenic mammary gland as a bioreactor for rhFVIII production. 15. Conference of Biotechnology 2006, Ceske Budejovice, Czech Republic, February, pp. 55-57.

CHRENEK P., VASICEK D., MAKAREVICH A., JURCIK R., SUVEGOVA K., BAUER M., PARKANYI V., RAFAY J., BATOROVA A., PALEYANDA R. K. 2005. Increased transgene integration efficiency upon microinjection of DNA into both pronuclei of rabbit embryos. Transgenic Res. **14**: 417-428.

FAN J., WATANABE T. 2003. Transgenic rabbits as therapeutic protein bioreactors and human disease models. Pharmacology & Therapeutics **99**: 261-282.

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. FASEB J. **14**: 2221-2229.

KARP L. E., SMITH W. D. 1975. Experimental production of aneuploidy in mouse oocytes. Gynecol. Invest. **6**: 337-341.

MARTINIAKOVA M., OMELKA R., CHRENEK P., RYBAN L., PARKANYI V., GROSSKOPF B., VONDRAKOVA M., BAUEROVA M. 2005. Changes of femoral bone tissue microstructure in transgenic rabbits. Folia biol. (Praha) **51**: 140-144.

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

PARKANYI V., CHRENEK P., RAFAY J., SUVEGOVA K., JURCIK R., MAKAREVICH A. V., PIVKO J., HETENYI L., PALEYANDA R. K. 2004. Aneuploidy in the transgenic rabbit. Folia biol. (Praha) **50**: 194-199.

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. Biol. Reprod. **71**: 340-347.