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PROCEEDINGS

Genetics – Short papers

STABILITY OF TRANSGENE TRANSMISSION IN THREE GENERATIONS OF TRANSGENIC RABBITS AFTER SINGLE OR DOUBLE PRONUCLEAR MICROINJECTION

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ABSTRACT

Several problems are commonly encountered with the production of transgenic rabbits: a low pregnancy rate, small litter size, cannibalism, mosaic founders incapable of germ-line transmission, a low transgene transmission rate and uncontrolled expression. One of the requirements for creating lines of transgenic animals creation is stable transgene transmission to offspring. In the present study, we demonstrate the transmission of the heterologous gene mouse WAP-human FVIII into F_1 and F_2 generations. Transgenic founder rabbits were generated by microinjection into a single pronucleus (single microinjection, SM) or into both pronuclei (double microinjection, DM). The rabbits were apparently normal and mating of founders with non-transgenic rabbits yielded litters of normal size (8 ± 0.40). PCR and Southern-blots demonstrated integration of the WAP-hFVIII gene in 35% or 44% of the SM or DM F_1 generation, respectively. Stability of transgene transmission to the F_2 generation was also confirmed (44% or 43%, resp.). Transgenic males and females from F_1 generation were bred to obtain homozygous animals. 73% and 77% of SM and DM offspring tested positive for the transgene. The number of copies integrated into the genome was estimated by Southern blot analysis. Both founders had one copy of the gene, which was transmitted to their transgenic offspring. Our results confirm successful integration and stable germ-line transmission of the WAP-hFVIII hybrid gene in both SM and DM lines and in three rabbit generations. This also indicates that double microinjection does not have any deleterious effects on transgene transgene integration and transmission in rabbits.

Key words: transgenic rabbit, human Factor VIII, transgene integration, transmission.

INTRODUCTION

Transgenic rabbits present an alternative way to produce therapeutic proteins in their mammary gland (BOZSE *et al*., 2003). The first transgenic rabbit was obtained approximately two decades ago, and factors which influences the efficiency of transgenesis have been addressed (HAMMER *et al*., 1985; VIGLIETTA *et al*., 1997; FAN and WATANABE, 2003). The greatest factor limiting the efficiency of the production of transgenic animals is the low rate of transgene incorporation into the genome of microinjected embryos. The other factor important in creating stable lines of transgenic animals for scientific analysis or for protein production is the stability of transgene transmission to offspring. Although pronuclear microinjection is the most successful method to produce transgenic animals, this technique results in random insertional transgenesis. The mechanism by which injected DNA integrates into a chromosome is unknown. DNA usually integrates at a single site on a chromosome but multiple integration can occur as well (WILMUT *et al*., 1991). Therefore, it is important to control transgene transmission through several generations. We have generated transgenic founder rabbits by microinjection into a single pronucleus (single microinjection, SM) or into both pronucleii (double microinjection, DM). The aim of this preliminary study was to investigate germ-line transmission of the mouse whey acidic protein (WAP)-human Factor VIII (hFVIII) hybrid gene in three generations of rabbits and in both SM and DM lines.

MATERIAL AND METHODS

- Transgenic rabbits were produced by microinjection of WAP-hFVIII gene construct into the male pronucleus (SM), or into both pronucleii (DM) of fertilized eggs from superovulated New Zealand White or California rabbits (CHRENEK *et al*., 2003). The gene construct was kindly provided by Dr. H. Lubon (American Red Cross, Maryland, USA).

- DNA was isolated from ear tissue of newborn animals and PCR was used to detect integration of the WAP-hFVIII gene (VASICEK *et al*., 2003). Analysis of WAP-hFVIII transgene integration and copy number estimation was carried out by Southern blotting, as described by PALEYANDA *et al*. 1997.

- Transgenic founders or F_0 generation animals were mated with non-transgenic rabbits of the same breed to obtain F_1 heterozygous offspring. Transgenic F_1 generation males were crossed with transgenic females from the same generation to obtain heterologous and homozygous F_2 generation animals.

RESULTS AND DISCUSSION

The integration frequency of mWAP-hFVIII into the genome of transgenic rabbits was 3.3% from single microinjection and 8.1% from double microinjection experiments (CHRENEK *et al*., 2003). Two lines of transgenic rabbits carrying the 2.5 kb murine whey acidic protein promoter, 7.2 kb cDNA of the human clotting factor VIII, and 4.6 kb of 3' flanking sequences of mWAP gene were studied. Line I was established after embryo transfer of SM eggs (Figure 1), and Line II, after embryo transfer of DM eggs (Figure 2). PCR analysis was used to detect integration of the WAP-hFVIII gene into the genome of newborn rabbits.

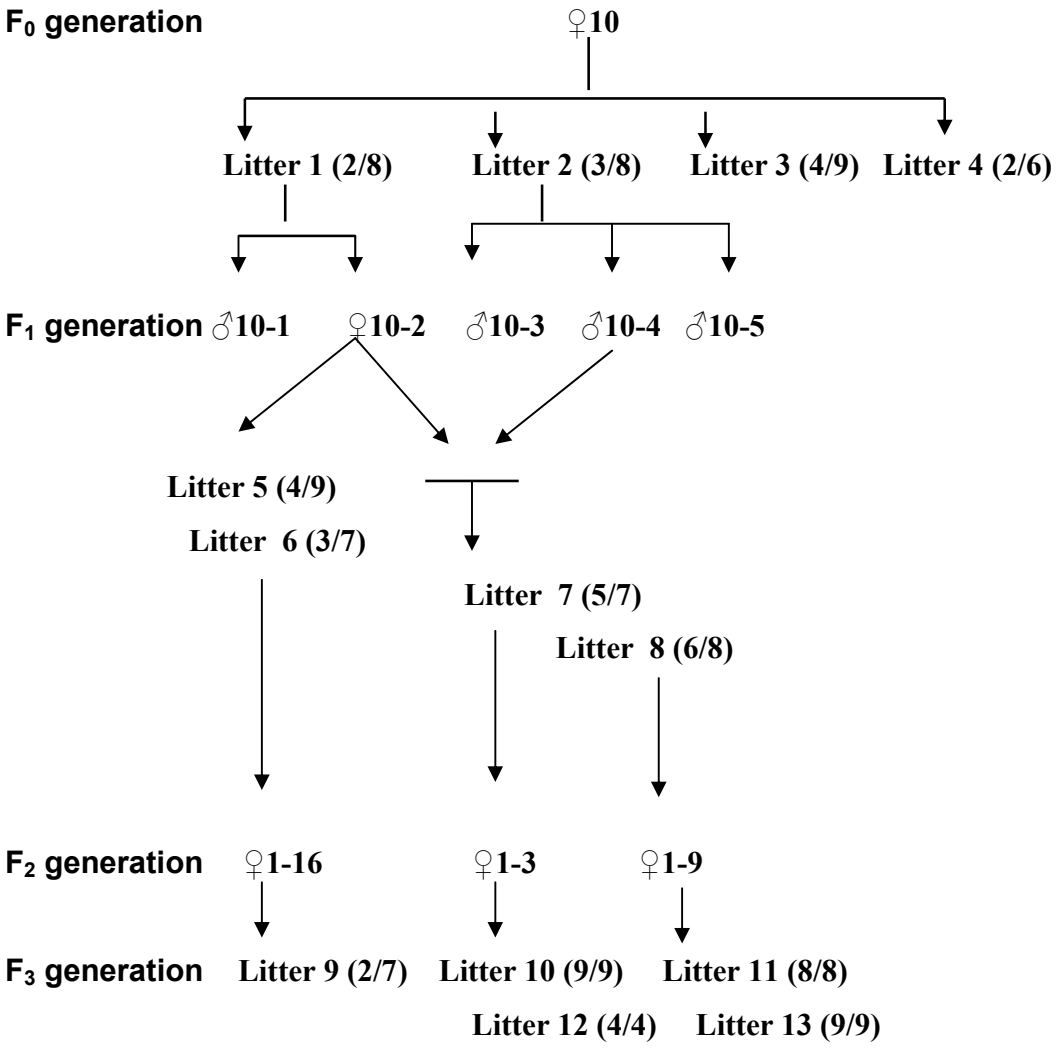

Figure 1. Family tree of transgenic Line I (SM), derived from WAP-hFVIII founder ♀10

The hemizygous founder rabbits were crossed with non-transgenic rabbits of the same breed to study transgene transmission. In the F_1 generation, the efficiency of transgene integration was 35% for SM and 44% for DM animals (Table 1).

The stability of transgene transmission from F_1 to F_2 generations, was 44% for SM and 43% for DM (Table 1). To obtain homozygous F_2 generation animals, transgenic males and females from F_1 generation were mated. 73% and 77% offspring in both lines (SM and DM) were transgenic.

Percental data, which were estimated by chi-square test between SM and DM lines were not statistically different (P<0.05).

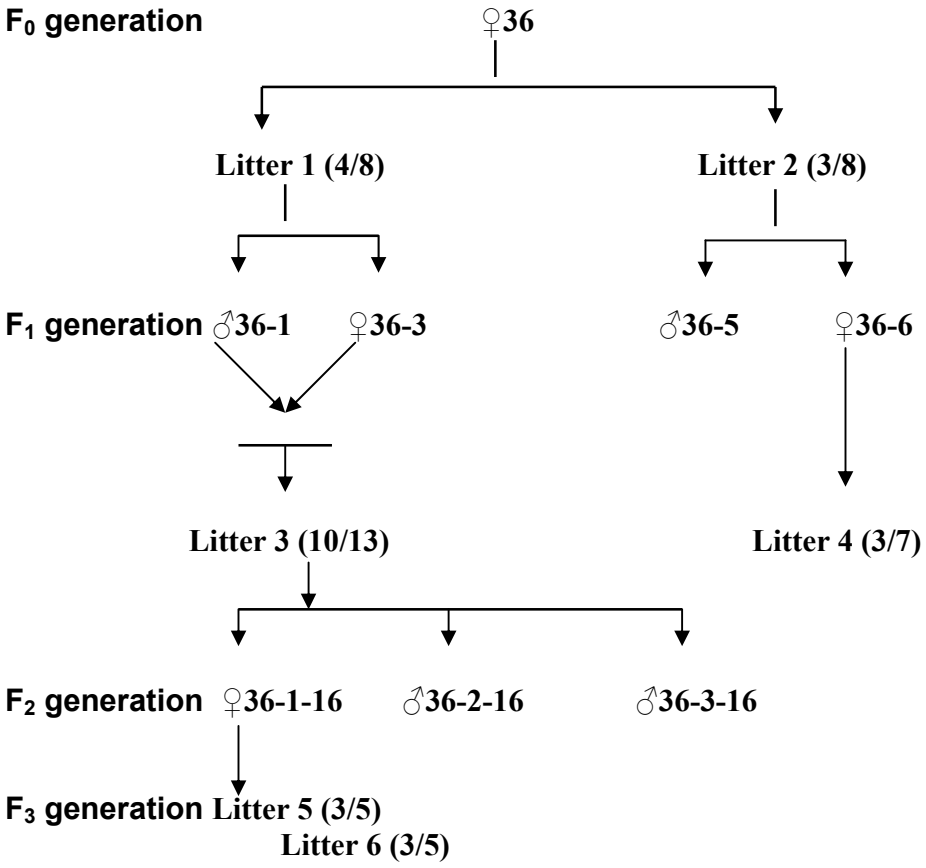

Figure 2. Family tree of transgenic Line II (DM), derived from WAP-hFVIII founder ♀36

Stability of transgene transmission in offspring is one of the requirements for transgenic animals used in the production of therapeutic proteins by mammary gland (VAN COTT *et al.*, 1997). Integration of foreign DNA into the embryonic

genome generally is a random event with respect to the chromosomal locus, and instability of transgene transmission could be expected. However, our preliminary results show stable transmission and correspond to data from other groups on stable transmission of other transgenes in mice, rabbits and pigs (CHEN *et al*., 2002; CHRENEK *et al*., 2002; VAN COTT *et al*., 1997).

Table 1. Stability of transgene transmission in three generations of transgenic rabbits

Generation	Single Microinjection, SM	Double Microinjection, DM
F_0 –hemizygous	1/30 (3%)	4/49 (8%)
F_1 –heterozygous*	11/31 (35%)	7/16 (44%)
F_2 –heterozygous*	7/16 (44%)	3/7 (43%)
F_2 –homozygous	11/15 (73%)	10/13 (77%)

*Data were not statistically different (P<0.05)

We determined the inheritance of the WAP-hFVIII transgene by analyzing the copy number per genome in founders, as well as in F_1 and F_2 offspring of both SM and DM lines, by Southern-blotting (Figure 3). Both founder 10 (Line I, SM) and founders 36 (Line II, DM) were estimated to have one copy of the transgene. Only one out of 8 F_1 offspring had an estimated 8 copies of the transgene (Figure 3, lane 10). F_1 animals transmitted one copy of stably integrated gene to their F_2 generation (data not shown).

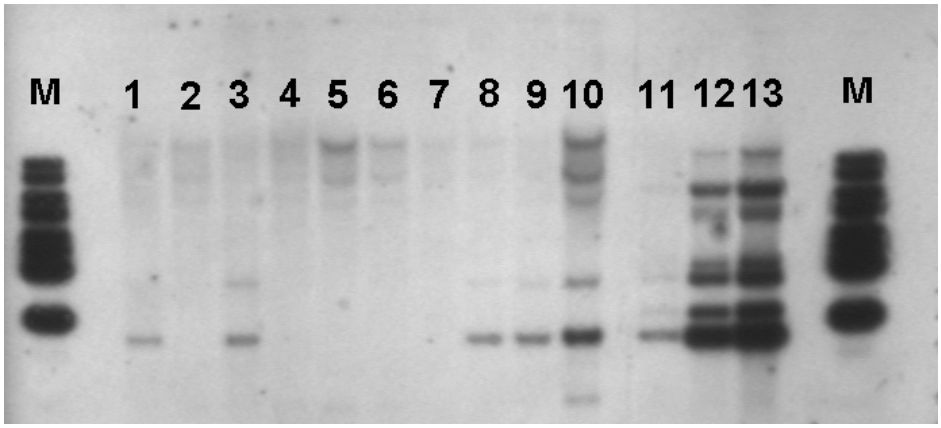

Figure 3: Southern blot analysis of DNA from rabbit ear tissue

M – marker (1kb DNA Ladder); Lane 1 – founder ♀10, (SM); Lane 2, 4, 7, – F1 generation heterozygous - (SM); Lane 3 – founder ♀36, hemizygous (DM); Lane 5, 6,– F1 generation (DM); Lanes 8, 9, 10 – ♂10-3, ♂10-4, ♂10-5 from F1 generation heterozygous, Lane 11 – 1 copy of gene construct; Lane 12 – 10 copies of gene construct; Lane 13 – 25 copies of gene construct.

CONCLUSIONS

The mouse WAP promoter-hFVIII cDNA transgene was transmitted stably by founders to F_1 and F_2 generation animals. Our results demonstrate that the transmission of the WAP-hFVIII transgene is stable, regardless of whether single or double microinjection was used to create the transgenic rabbit lines.

ACKNOWLEDGEMENTS

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