SZENT ISTVÁN UNIVERSITY

Animal Husbandry Doctoral School

TRANSGENIC RABBIT GENERATION WITH LENTIVIRAL AND SLEEPING BEAUTY TRANSPOSON MEDIATED TRANSGENESIS

Thesis

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1. INTRODUCTION AND GOALS

Transgenesis is a part of biotechnology, which covers a set of suitable methods to modify the genome of living organisms. Transgenic techniques, despite their dual perception are today's major fields in the study of diseases. They are also important tools for the production of recombinant proteins and for testing the functions of genes. Today, the transgenic organisms coming from our laboratories to the agriculture are and - intentionally or unintentionally - crept into our lives. One can find them in our fields, on our plates, but even among our pets.

Rabbit stands between laboratory rodents and cattle. As a laboratory modell, rabbit is very useful, because rabbit is physiologically closer to humans than other laboratory models, like mouse or rat. Rabbit is mainly used in a lot of reproduction study, and to test some micromanipulation techniques (for examlpe for pronuclear injection or embryo splitting).

Genetic modification of the rabbit exists a long time ago, but methods in its infancy in terms of efficiency are used to produce a transgenic rabbit model. Efficiency of transgenic rabbit lines described in the literature, the creation of a few percent, which not only involves the temporal and material disadvantage, but raise animal welfare issues.

My aim was to research a working and easy-to-apply method with greater efficiency for creating transgenic rabbits. In my thesis I present two second-generation technology, which is capable of highly efficient transgenesis in this species.

2. MATHERIALS AND METHODS

We used the Hycole breed for generating transgenic rabbits. The animals were maintained in a conventional animal house in individual cages at 18±3°C, 12 hours light, with *ad libitum* feeding.

The Hycole donor female rabbits at 3-4 months of age with the prescence of at least 3.5 kg of body weight were introduced intramuscularly with 120 IU PMSG. The rabbits were treated intravenously with 180 IU hCG 72 hours after the PMSG injection. Simultaniusly with the hDG threatment, the rabbits were artificially inseminated with fresh Hycole semen. The day after insemination the embryos were flushed out from the oviduct of the donor rabbits with PBS media supplemented by 20% FCS. The embryos were stored in media covered with mineral oil, in 38,5°C and 5% CO_2 enviroment until microinjection.

We used two basic methods of microinjection in our experiments: one is the pronucleus injection used for the Sleeping Beauty transposon system, on the other hand, the lentiviral construct was injected to the perivitellinar space of the embryos.

The embryos derived from the oviduct of the donor animals were selected. The embryos with suitable quality and visible pronucleus were pipetted into a media droplet placed on a depressed glass slide. The media droplet was covered with mineral oil to avoid the concentration change caused by the evaporation. The slide was gently placed into the microscope (Olympus IMT-2) and the micromanipulation was carried out with micromanipulator arms (Narishige) and glass capillaries.

The manipulated embryos were transferred into the oviduct of pseudopregnant recipient females. The transgenic offsprings were selected with UV light and with transgene specific PCR.

For the determination of the integration site and copy number standard molecular techniques were used. We examined the prescence of the transgene in different tissues with molecular and with microscopyc methods also.

In both approaches, we selected 4 founders for breeding.

3. RESULTS

3.1.Lentiviral based transgenesis

The lentiviral vector was injected into the perivitellinar space of the one cell rabbit embryos as the lentivirus is capable to plough through the membranes. The major advantages of the lentiviral method are that, the perivitellinar injection is less destructive for the embryos and easier to perform than the pronuclear injection. This is supported by our experiments as the 81% of the recipient does were gave birth and the 29.8% of the transferred embryos were borned.

In our case, all of the founders (28 individuals) have shown mosaic pattern. The four – seemingly less mosaic – founders have been choosen for breeding produce 215 F1 progenies. None of the borned offspring were expressing the transgene. In three cases of 215 we could detect the transgene with PCR. Two – stillborn – offspring carried the transgene, but we did not detect any expression. This silencing event may be caused by various epigenetic modifications, that has been described in case of lentiviral transgenesis in other mammals as well. All the tissues of one 13.5 day embryo were expressing the transgene at protein level. Thus, the SIV lentiviral construct has been introduced to the germline even thougt this embryo did not borned.

3.2.Transposon based transgenesis

In our experiments we microinjected the components of the Sleeping Beauty transposon system to the pronuclei of one cell stage rabbit embryos. 10% of the microinjected and transferred embryos were borned, 40% of the recipient does were calved. 15%, 7 individuals of the total borned pups were transgenic. All of the founders expressed the transgene, we did not observe gene silencing. Likewise in case of lentiviral transgenesis mosaic pattern could be detected in all founders, however all the four founders selected for the breeding inherited the transgene.

Both techniques involved mosaicsm, which is rather due to the rabbit's fast embryonic development, than the speed of the technology, in case of the Sleeping Beauty transposon system. This is prooven by the fact that far fewer cases of mosaicism occur in transgenic mice created with Sleeping Beauty transposase system, than in case of rabbit.

The SB 3 BT transgenic founder was selected for creating a homozygous line after the extensive characterisation of the integration into this individual. In this line we did not experienced any silencing events, all of the progenies were healthy and their physiological parameters did not differ from their littermates of the same age. The prescence of the transgene in different sections of tissues were tested and gene silnecing was not observed however, gene silencing described in mice.

4. NEW SCIENTIFIC RESULTS

1. I created the first transgenic rabbit in the world with transposon-based transgenesis.

2. I attended in the creation of the world's first transgenic rabbit by lentiviral based transgenesis.

3. The founders created with lentiviral based transgenesis are mosaic, but the transgene can be integrated and expressed in all the three germ cell layers. I found that the inheritance of the transgene using lentiviral technique has very low efficiency in case of rabbit.

4. I managed to create a homozygous transgenic rabbit line expressing a riporter gene in all tissues, which has so far been lacked from the litriture and essential for our other experiments.

5. With the Sleeping Beauty transposon system the transgenic founders though were slightly mosaic, but all of them inherited the transgene in a mendelian pattern and gene silencing did not occur. I found that the Sleeping Beauty transposon system is capable for creating transgenic rabbits, what carry the transgene in low copy number, and the intergation sites do not hits genes.

6. With the comparism of lentiviral and transposon based methods I determined that the Sleeping Beauty transposon system exceeds the methods, that has been used so far in rabbit in terms of efficiency and usability.

5. SUMMARY

There is an indisputable significance of the rabbit as a modell animal for life sciences. This species is commonly used to model human diseases, in particular for the production of recombinant proteins or reproduction studies, because the housing and breeding of rabbit is relatively cost-effective. Because of it's extensive use the rabbit is a well-studied laboratory animal. Although there are plenty methods, which are refined in case of other laboratory animals such as mouse or rat, but in case of rabbit they have low efficiency.

Despite that the rabbit is a very important laboratory animal modell for study of several human diseases, the transgenesis of the rabbit is inefficient. As it was absent form the literature, my aim was to develop a novel, efficient procedure to support the creation of a transgenic rabbit line.

In my work, two second-generation transgenic techniques have been used, these methods have already proven their effectiveness in other species. With the application of the lentiviral technology we could create transgenic founders. The efficiency of the transgenesis was 32%, which is considered as a very high value when compared to our previous treatment based on conventional DNA microinjection. All the founders shown highly mosaic pattern. Despite the huge number of founders and F1 generation pups, we did not manage to establish a transgenic line. One possible explanation could be the mozaicism or the gene silencing which is inherent in the lentiviral systems. However, inheritance of the transgene was demonstrated in three cases, thus the germline transgenesis was successful.

After using the lentiviral technology we applied the Sleeping Beauty transposon system. This transposon mediated method has been tested on a number of laboratory and farm animals, but not on rabbit. In case of Sleeping Beauty transposon mediated technique the transgenesis efficiency proved to be 15%. After the fully comprehensive characterisation of the founders we selected four animals. With all the four selected founders we were able to establish a transgenic line. With inbreeding we developed a homozygous line, which we maintain in our animal facility and use for other studies.

Although, with both technologies we achieved efficient transgenesis rates in case of rabbit, the Sleeping Beauty system was still more usefull. With this two second-generation method we could create transgenic rabbits in a much faster and less expenditure-needed way than with conventionally transgenesis. As a result of my work we introduced the application of the Sleeping Beauty transposon system to our laboratory, which has been successfully applied in many cases ever since.

If we review the conventional and this two second-generation methods a highly visible development unfolds before our eyes in the field of transgenesis. A 30-year trend, while the transgenesis become faster, more secure and cost-effective, anticipates the growing availabity of the transgenic animals in fields of fundamental and applied research. This progress can also be observed in rabbit with a slight delay, if we compare it with other frequently used laboratory animals (rats, mice). Our group played a large role in the advancement of rabbit transgenesis with the first administration of the lentiviral and the Sleeping Beauty transposon mediated transgenesis.

Animal protection is an important issue also in a case of laboratory animals. The second-generation techniques used in my experiments contribute to realise the 3R rule by their effectivenes in decreasing the number of animal subjects.

Rabbit, which is often used as an animal model of human diseases and has an undeniable role in the production of reconmbinant proteins, is becoming a more frequent target of the transgenesis. My work grounds a simple and efficient way of the genetic manipulation of this species, which has so far been lacked in the scientific literature.

In the future I would like to establish a rabbit line with Sleeping Beauty mediated transgenesis, which carries a single riporter gene as a transgene with recombinase mediated cassette exchange sites. Thereafter the reporter gene may replaced to another gene, which has a fundamental reasearch importance.

6. PUBLICATIONS

1. First author paper

   The FASEB Journal vol. 27 no. 3 930-941
   Transposon-mediated Transgenesis, Transgenic Rescue, and Tissue-specific Gene Expression in Rodents and Rabbit
   Kettler K #, Geurts A #, **Hoffmann O** #, Mátés L., Landae V, Hiripi L, Moreno C., Lazar J, Bashir S, Zideke V, Popova E , Jerchow B, Becker K, Devaraj A, Walter I, Grzybowksi M, Corbett M, Filho RA, Hodges MR, Bader M, Ivics Z, Jacob HJ, Pravenec M, Bősze Zs, Rülicke T and Izsvák Z # contributed equally
   IF=5,712

2. Lectured papers

   PLoS One. 2012;7(1):e28869. Epub 2012 Jan 11.
   Characterisation of the Rabbit Neonatal Fc Receptor (FcRn) and Analyzing the Immunophenotype of the Transgenic Rabbits That Overexpress FcRn Catunda
   Lemos AP., Cervenak J., Bender B., **Hoffmann OI**., Baranyi M., Kerekes A., Bősze Zs., Hiripi L., Kacskovics I.
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   Transgenic Res. 2010 Oct;19(5):799-808. Epub 2010 Jan 13.
   Transgenic rabbit production with simian immunodeficiency virus-derived lentiviral vector
   Hiripi L., Negre D.., Cosset FL., Kvell K., Czömpöly T., Baranyi M., Gózca E., **Hoffmann OI**., Bender B., Bősze Zs.
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3. Lectures in international conferences

   Transzgénikus nyúl létrehozása lentivírus alapú transzgenezissel
   Hoffmann O.I., Hiripi L., Ivics Z., Izsvák Zs., Mátés L., Bősze Zs.
   TUDOC-2010, Kárpát medencei doktoranduszok nemzetközi konferenciája, Gödöllő, 2010

   Sleeping Beauty transgenesis in rabbit
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   4th International Rabbit Biotechnology Meeting, 30th June – 1st July 2011, Hungarian Academy of Sciences, Budapest

   IgG binding FcRn transgenic rabbits created through BAC transgensis
   Bősze Zs., Hiripi L., **Hoffmann O.I.,** Kerekes A., Bender B., Kacskovics I.
   4th International Rabbit Biotechnology Meeting, 30th June – 1st July 2011, Hungarian Academy of Sciences, Budapest

   Alternative transgenic methods in rabbit
   Hiripi L., **Hoffmann O.I.,** Bősze Zs.
   RGB-Net Meeting, 28-30 March 2012, Bologna, Italy

4. Lectures in hungarian conferences

Az ABCG1 transzporter túltermelésének hatása transzgénikus egér embriókban
Hoffmann O.I., Hiripi L., Bősze Zs.
1.Gödöllői Állattenyésztési Tudumányos Napok, Gödöllő, 2008

Lentivírus alapú transzgenezis nyúlban
Hiripi L., Kvell K., Czömpöly T., **Hoffmann O.I.,** Baranyi M., Cosset F-L., Negre D., Bodrogi L., Gócza E., Bősze Zs.
MBK Napok, Gödöllő, 2009

Transzgénikus nyulak létrehozása lentivírus vektorokkal
Hiripi L., Kvell K., Gócza E., Czömpöly T., Bodrogi L., **Hoffmann O.I**., Baranyi M., Bősze Zs.
VIII. Magyar Genetikai Kongresszus és XV. Sejt- és fejlődésbiológiai Napok, Nyíregyháza, 2009

IgG kötő Fc receptort túltermelő transzgénikus nyúlmodell előállítása
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MBK Napok, Gödöllő, 2009

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Szarvasmarha szabályozó SNP-k tesztelése in vivo egér modellben
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MBK Napok, Gödöllő, 2011

5. Posters in international conferences

Adaptation of the lentiviral technology to produce transgenic rabbit
Hiripi L., Kvell K., Czömpöly T., Baranyi M., Gócza E., Bender B., **Hoffmann O.I.**, Bősze Zs.
Chromatin domains and insulators, Baeza, Spain, 9th-11th November 2009, Baeza, Spain

Cloning and characterization of the rabbit neonatal Fc receptor
Lemos Ana Paula Catunda, Judit Cervenak, **Orsolya Hoffmann**, Anita Farkas, László Hiripi, Imre Kacskovics
ISAG 2010, June, Edinburgh UK, Poster

Rabbit transgenesis with Sleeping Beauty transposon system
Hoffmann O.I., Hiripi L., Kerekes A., Ivics Z., Izsvák Zs., Mátés L., Bősze Zs.
75th anniversary of Albert Szent-Györgyi's Nobel Price award, Szeged, 22-25 March, 2012

Homozygous transgenic rabbit line expressing Venus reporter gene created by Sleeping Beauty transposon system
Hoffmann O.I., Hiripi L., Ivics Z., Izsvák Zs., Mátés L., Bősze Zs**.**
CEELA, II.Közép- és Kelet-Európai Laborállat-tudományi Konferencia, Budapest, 2012. június 2.

6. Posters in hungarian conferences

A nyúl neonatális Fc receptor génjének izolálása és jellemzése
Lemos A.P.C., **Hoffmann O.I**., Hiripi L., Cervenák J., Kacskovics I., Bősze Zs.
VIII. Magyar Genetikai Kongresszus és XV. Sejt- és fejlődésbiológiai Napok, Nyíregyháza, 2009

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2. Gödöllői Állattenyésztési Tudumányos Napok, Gödöllő, 2008

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