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Short communication

Transgenic mouse model integrating siRNA targeting the foot and mouth disease virus

Wang Pengyan[a,b], Jiang Jianjun[a,b], Li Ning[c], Sheng Jinliang[a,b], Ren Yan[a,b], Chen Chuangfu[a,b,*], Guo Zhiru[a,b]

[a] Key Laboratory for AgroBiotecnology of Shihezi University, Shihezi, Xinjiang 832003, PR China
[b] College of Animal Science and Technology, Shihezi University, Shihezi, Xinjiang 832003, PR China
[c] State Key Laboratory for AgroBiotecnology of China Agricultural University, Beijing 100094, PR China

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ABSTRACT

We have constructed 2 small interfering RNAs (siRNAs) specifically targeting homogenous 3D and 2B1 regions of 7 serotypes of the foot and mouth disease virus (FMDV) and tested the ability of siRNAs to inhibit virus replication in baby hamster kidney (BHK-21) cells and suckling mice. In this study, we generated transgenic mouse models integrating short hairpin RNA (shRNA) targeting microinfected FMDV. When examined at the 7th passage in transgenic mice, the target gene was still found by PCR to be integrated in the genome. Compared to the control mice, the transgenic mice showed only slightly abnormal pathology when they were infected with the FMDV serotype Asia 1. The number of viruses in the tissues of the transgenic mouse was very low and in some tissues no virus could be detected by immunohistochemistry.

RNA interference (RNAi) has been extensively used for sequence-specific silencing of genes in mammalian cells. The latest important breakthrough in the application of RNAi technology was achieved in experiments demonstrating RNAi-mediated gene repression in mice and rats. After more than 2 decades of research on mice that was aimed at developing and continuously improving transgenic and knock-out technologies, the generation of RNAi-knock-down mice represents a valuable new alternative for studying gene function in vivo. Furthermore, RNAi holds promise as a fast and cost-effective approach to study mammalian gene function in vivo and as a novel therapeutic approach. In this study, we generated a transgenic mouse model that integrated short hairpin RNA (shRNA) targeting the foot and mouth disease virus (FMDV). This is the first report to show the use of RNAi in conferring enhanced resistance to FMDV in transgenic animals, and the results offer insight into the use of RNAi in animal breeding for disease resistance.

In order to generate transgenic mice two shRNA-expression pSi-FMD2 and pSi-FMD3, constructed in the lab (Pengyan et al., 2008), were prepared in large quantities and digested with *Hin*d III and *Sac* II. The small fragment containing the siRNA was diluted to 1.4 μg/ml and 5 μl/tube and preserved at −20 °C after recovery and purification. Four-to-six-week-old suckling (Kunming white) mice were used for breeding and embryo transplantation. The generation of mice, collection of zygotes, microinjection and embryo transplantation were carried out as described by Xiaoli et al. (1995). Two small fragments (containing the siRNA) were microinjected into the male pronucleus of the zygote. The fragment of pSi-FMD2 was microinjected into 506 zygotes, which were transplanted into the oviducts of 23 mice; of these 17 became pregnant and gave birth to an offspring of 104. The fragment of pSi-FMD3 was microinjected into 469 zygotes, which were transplanted into the oviducts of 22 mice; of these 9 became pregnant and gave birth to an offspring of 55. The genomic DNA was extracted from tail clippings (1–1.5 cm) from all the 21-day-old offspring. We used 1 μl template DNA for PCR. siRNA-expressing plasmids were used as the

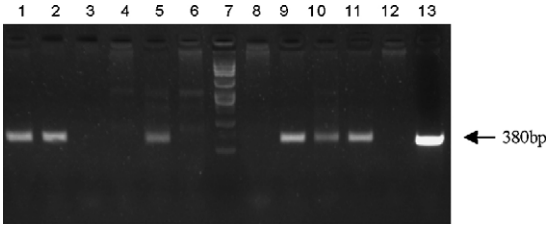

Fig. 1. PCR of G0 the genomic DNA of transgenic mice. Lane 7: 100 bp ladder marker, lane 12: negative control, lane 13: positive control, lanes 1, 2, 5, 9–11: transgenic mice, lanes 3, 4, 6 and 8: negative results.

* Corresponding author at: Key Laboratory for AgroBiotecnology of Shihezi University, Shihezi, Xinjiang 832003, PR China.
E-mail addresses: ccf-xb@163.com (C. Chuangfu), guozhiru@yahoo.com (G. Zhiru).

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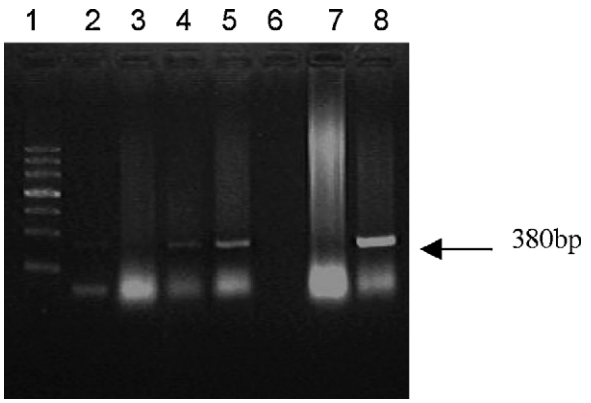

Fig. 2. PCR of the genomic DNA of G7 transgenic mouse. Lane 1: 100 bp ladder marker, lane 8: negative control, lane 6: positive control, lanes 2, 4 and 5: transgenic mice, lanes 3 and 7: negative result.

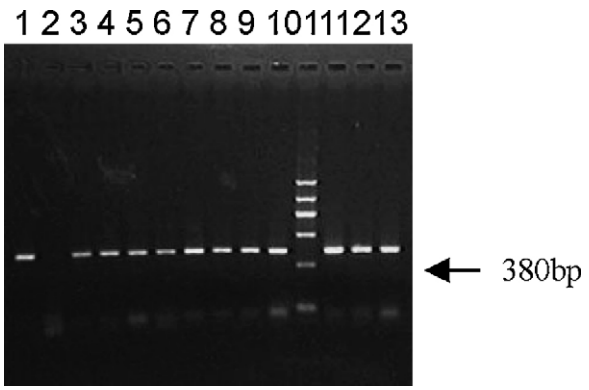

Fig. 3. PCR of the genomic DNA of G5 transgenic mouse. Lane 1: positive control, lane 2: negative control, lane 11: 100 bp ladder marker, lanes 3–14: transgenic mice.

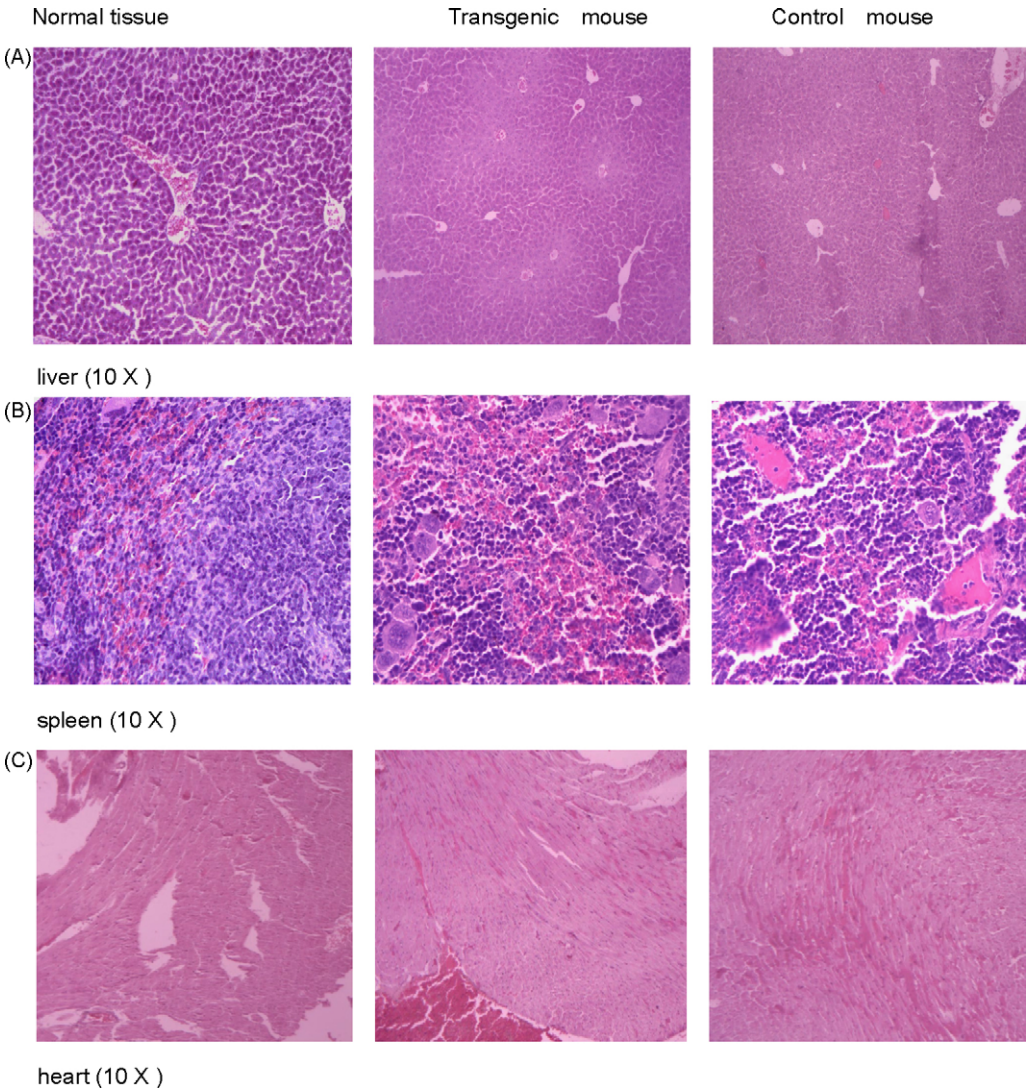

Fig. 4. HE staining of different pathological sections of tissues from transgenic and control mice. (A) HE staining of liver sections revealed that there was severe hepatic congestion in control mice; however it was normal in transgenic mice, (B) HE staining of spleen sections revealed that the number of splenic corpuscles in the transgenic mice was higher, and macrophage epithelioid cell node in the junction of white pulp and red pulp had increased significantly, (C) HE staining of heart sections revealed that there were almost no change in transgenic mice, however there were severe cardiac muscle waxy necrosis in control mice.

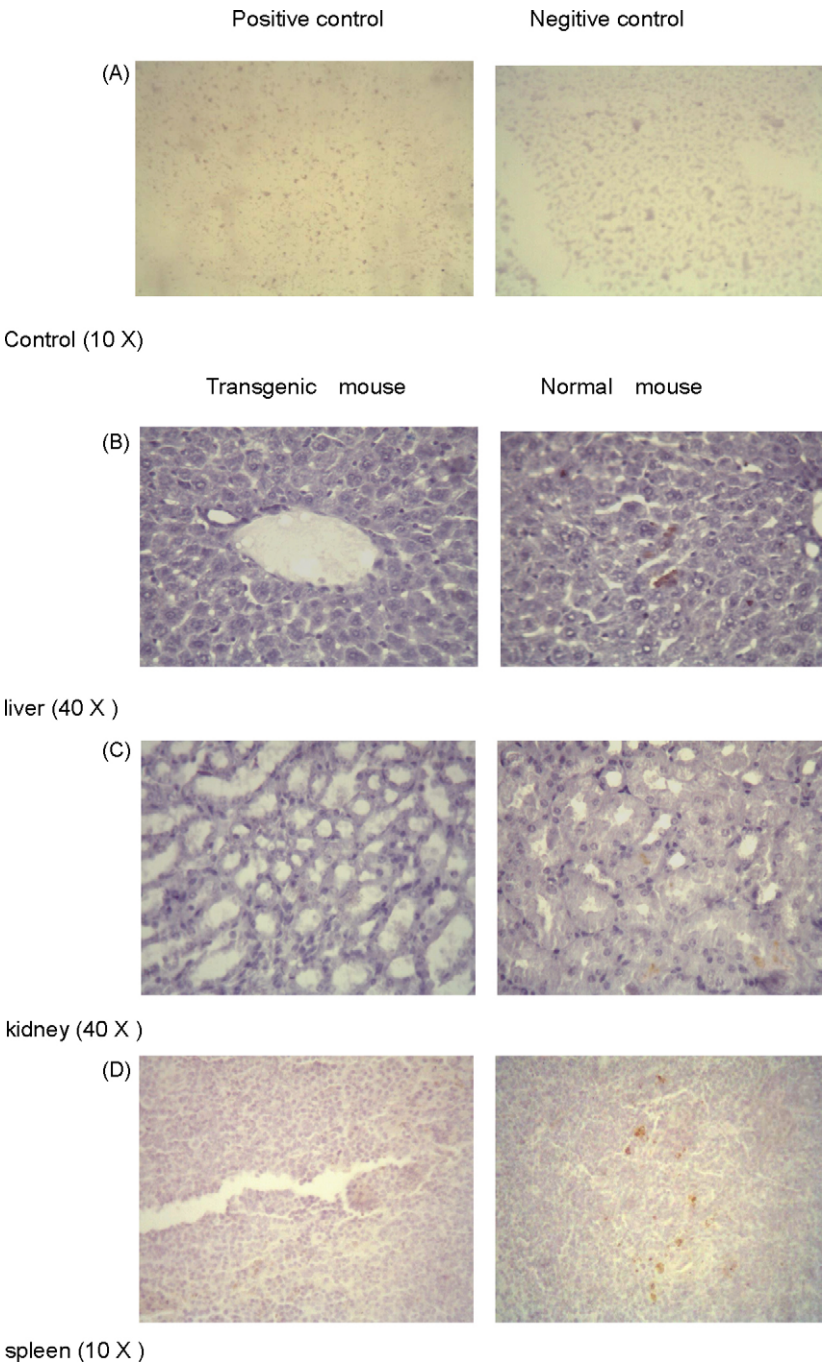

Fig. 5. Results of immunohistochemistry. (A) Immunohistochemistry results of FMDV on BHK-21 cell. Positive control refers to BHK-21 cells infected with FMDV. Negative control refers to normal BHK-21 cells. (B, C and D) Immunohistochemistry results of liver, kidney and spleen of transgenic mice and normal mice. There were no virions in the liver and kidney of transgenic mice and few virions in the spleen of transgenic mice.

positive control and the genomic DNA of normal mice as the negative control. The sequences of up-stream and down-stream primers were as follows: F2: 5′-TCCCAGAACACATAGCGACA-3′; and R2: 5′-CAGAAAGCGAAGGAGCAAAG-3′. The PCR program was as follows: 5 min at 94 °C; 30 cycles of 30 s at 94 °C, 45 s at 58 °C, and 30 s at 72 °C; and 7 min at 72 °C. We used 5 μl of the PCR product for 1% agarose gel electrophoresis. The PCR results of 159 samples of genomic DNA showed that there were 6 positive transgenic mice (Fig. 1). The rate of integration of the transgene was 20–30% in G1–G7 animals (Fig. 2). When mated between two transgenic mice, 100% of the offspring were transgenic mice (Fig. 3). The offspring has demonstrated no abnormalities to date.

To further test the anti-FMDV activity of the siRNAs, six G1 positive transgenic mice were challenged with 0.5 ml of 100 LD_{50} Asia1 FMDV via intraperitoneal injection. Saline-treated mice were used as the control. Seventy-two hours after the viral challenge, the mice were killed by exsanguination from the fossa orbitalis and the heart, liver, spleen, lungs, kidneys and muscles were excised and fixed in 10% formalin. Routine pathological sections of these tissues were prepared and the pathological changes were observed under a microscope. The resistance of transgenic mice to FMDV infection was evaluated by an immunohistochemistry assay. The results of hematoxylin-eosin (HE) staining of different pathological sections revealed that compared to the control mice, the transgenic

mice showed slightly abnormal pathology. In particular, the number of splenic corpuscles in the transgenic mice was higher, and macrophage epithelioid cell node in the junction of white pulp and red pulp had increased significantly (Fig. 4).

FMDV could be detected by immunohistochemistry in the liver, spleen, and kidneys of the control mice. However, in the transgenic mice, FMDV could be detected only in the spleen and the liver, and the number of viruses was smaller than that seen in the control mice (Fig. 5).

There have been several studies on the inhibition of FMDV replication by siRNA at the cellular level (Kahawa et al., 2004; de los Santos et al., 2005; Liu et al., 2005). In this study, we generated a transgenic mouse model that integrates siRNA targeting the FMDV. These mice have a siRNA expression box, which can be transmitted to the offspring through the germline. Six transgenic mice and six normal control mice were infected with FMDV in the same way. Routine pathological examinations of the tissues of the transgenic mice showed only slightly abnormal pathology. At the same time, the results of immunohistochemistry showed that there were few or no viruses in each tissue of the transgenic mice. As shown in our previous study (Pengyan et al., 2008), the two siRNAs could trigger an antiviral response in BHK-21 cells infected with FMDV, but they could not completely inhibit virus replication. So in this work, it is possible that viruses were present in the tissues, but at a concentration below the threshold of detection by immunohistochemistry. However, compared with control mice, the number of splenic corpuscles in the transgenic mice was higher, and macrophage epithelioid cell node in the junction of white pulp and red pulp had increased significantly. It was presumed that after the entry of FMDV into the body, the siRNA targeting the FMDV inhibited the replication of FMDV at some extent, thus reducing the virus number. Thus, the transgenic mice generated in our experiment could effectively resist FMDV infection.

The technique of microinjection has been applied commonly since Gordon used it for introducing a gene into the zygote of a mouse (Gordon et al., 1980). It was reported (Hogan et al., 1994) that when microinjection was performed by skilled researchers, about 60% of zygotes survived. Additionally, there is no limitation of the size of DNA that can be introduced, and the foreign gene was confirmed to be integrated in the genome of 10–40% of the mice generated. Because the gene is introduced into the pronucleus it will produce less chimera. It is convenient to analyze G0 transgenic animals and establish new transgenic strains. In this study, each gene was microinjected into about 500 zygotes. Some zygotes died before transplantation. After the zygotes were transplanted into recipient mice, 17 and 9 mice became pregnant and produced an offspring of 104 and 55, respectively (see supra). At last together there were 6 transgenic mice.

It is apparent that introduction of favorable target genes into recipient animals will offer an effective way for improvement of varieties of domestic animal breeds. However, it cannot replace traditional breeding and should only be limited to the utilization of beneficial genes in domestic animal breeds. Scientists have obtained some materials of economic value from transgenic fishes, fowls and pigs by the transgenic technique. The success of transgenic anti-PrP^{SC} cattle could offer a new strategy for the prevention of bovine spongiform encephalopathy. The generation of transgenic mice or RNAi-knock-down mice is not as difficult as many people have thought. Mice created in such ways have a clean genetic background, which makes the phenotype analysis highly reliable. Furthermore, two or more RNAi vectors could be microinjected simultaneously, which would lead to double or multiple inhibition (Kittler and Buchholz, 2003). Theoretically, RNAi-knock-down could achieve 1–99% inhibition, an apparent advantage over the gene knock-out approaches from procedural perspectives. So far, there have been few studies on animal breeding for disease resistance in mice or other animal species using the RNAi-based knock-down approaches. Although RNAi-knock-down mice models have some limitations, they will be valuable for studies on screening important genes for animal breeding or other purposes in the postgenomic era.

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