PIBB 生物化学与生物物理进展
Progress in Biochemistry and Biophysics
2009, 36(7): 872~879
www.pibb.ac.cn

RNAi-mediated Stable Silencing of *HGPRT* Expression in Rabbit Fibroblasts and SCNT Embryo Production*

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Abstract The hypoxanthine-guanine phosphoribosyl transferase (HGPRT) gene mutation is responsible for gouty arthritis, kidney stone, and Lesch-Nyhan Syndrome (LNS). It has been reported that the expression of *HGPRT* is decreased or even absent in these diseases. Rabbits are an ideal model for studying the pathology of these diseases. Therefore, the development of an *HGPRT*-knockdown rabbit model will be highly beneficial in such studies. Stable *HGPRT*-knockdown transgenic fibroblast lines were generated by transfecting rabbit fibroblasts with RNA interference (RNAi) plasmids. Polymerase chain reaction (PCR) analyses indicated that the average positive rate was 83.3%. The mRNA and protein levels of *HGPRT* in the transgenic fibroblast lines were significantly lower than that in the control. Transgenic rabbit blastocysts were derived after performing nuclear transfer. The results show that RNAi can be used to stably knock down expression of the *HGPRT* in rabbit fibroblasts and further improvements in related technologies will facilitate the use of this method for the generation of *HGPRT*-knockdown rabbits.

Key words RNA interference, rabbit fibroblast, HGPRT, nuclear transfer
DOI: 10.3724/SP.J.1206.2008.00731

The hypoxanthine-guanine phosphoribosyl transferase (HGPRT), an enzyme that converts hypoxanthine and guanine into their respective 5′-mononucleotides, plays an important role in the metabolic salvage of purines in mammalian systems. HGPRT deficiency is associated with uric acid overproduction and a continuum spectrum of neurological manifestations. However, there is no effective animal model for studying HGPRT-associated diseases. Kuehn *et al.*[1] produced HPRT-deficient mice by using homologous recombination (HR) in mouse embryonic stem cells (ES), and Engle *et al.*[2] bred the nonfunctional adenine phosphoribosyl transferase (APRT) allele into an HPRT -deficient mouse and generated HPRT-APRT-deficient mice. However, these models did not exhibit any anatomical defects or spontaneous behavioral abnormalities. These studies indicated that mice are not a suitable model for studying the behavioral abnormalities associated with these diseases. However, the development of other suitable mammalian models for analyzing the developmental and pathological mechanisms of various diseases has been hindered by the unavailability of germline-competent ES cell lines of other mammalian species. The recently developed somatic cell nuclear transfer (SCNT) technology [3] allows the generation of transgenic animals without gene targeting in ES cells. Rabbits are medium-sized animals that can be easily bred at a comparatively lower cost, and they are considered as the preferred animal model for studying human diseases. Furthermore, rabbits have been successfully cloned from freshly isolated cumulus cells (Chesne *et al.*, 2002) and adult male fibroblasts (Li *et al.*, 2006)[4, 5].

*This work was supported by grants from Important Project of Basic Research for Science and Technology in Shanghai Government (08JC1413900), Agricultural Development by Science and Technology Program in Shanghai Government (2006)5-3 and Shanghai Leading Academic Discipline Project(S30201).
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Received: October 23, 2008 Accepted: January 9, 2009

We have been conducting SCNT experiments on rabbits for years. Therefore, we were interested in using SCNT to generate *HGPRT*-knockdown rabbits that could serve as a model for studying *HGPRT*-associated diseases.

RNA interference (RNAi) is a sequence-specific, gene-silencing phenomenon that is mediated by double-stranded RNA [6]. The introduction of short hairpin RNA (shRNA) into living cells is a potent tool for gene-function analysis [7]. Miyagishi *et al.*[8] demonstrated the feasibility of using shRNA-expressing vectors in efficient suppression of *in vivo* and *in vitro* expression of the target genes. More recently, this system was found to show a high efficiency in silencing superoxide dismutase1 [9], Dnmtls [10], and *PRNP* [11] in mammalian cells. In comparison with other RNAi methods, the vector-based shRNA system represents an inexpensive and uncomplicated approach for inhibiting persistently expressing genes [12 ~14]. More significantly, Golding *et al.*[15] used RNAi-based techniques to create a cloned transgenic goat fetus with dramatically reduced expression of PrP, and the analysis revealed a significant (> 90%) decrease in PrP expression levels in brain tissues. These results demonstrate that it is possible to produce the genetically modified models by SCNT after stable suppression of genes in *in vitro* cultured cells.

In the present study, we constructed *HGPRT* shRNA-expressing vectors and established stable shRNA-expressing rabbit fibroblast lines. Our results indicate that the *HGPRT* mRNA and protein levels in the transgenic fibroblast lines were significantly lower than that in the negative control. The nuclear transfer (NT)-embryo was developed to the blastocyst stage. This approach provides a simple alternative to the traditional knockout method for exploring biological function. Most importantly, this system suggests the possibility of generating the expected rabbit-disease model by using RNAi and SCNT.

1 Materials and methods

1.1 Construction of *HGPRT* shRNA expression plasmids

The target sequences of the shRNA-vectors for the *HGPRT* gene (GenBank number: EF219063) corresponded to the sequence sites 378～398, 519～539, and 594～614. The *HGPRT* shRNA expression plasmids were generated by designing and chemically synthesizing 3 pairs of oligonucleotide strands (Oligo1～Oligo6). Another pair of scrambled sequences (Oligo7 and Oligo8), which had no matches in the rabbit genome, were synthesized as negative controls. All the oligonucleotides were obtained from Shanghai Invitrogen Biotech Co. Ltd. (China). These oligonucleotides were annealed to produce double-stranded oligonucleotides; the double-stranded nucleotides were subcloned downstream of the U6 promoter in linearized PRNAT-U6.1/Neo (GenScript Corporation, USA) by using *Bam*HⅠ and *Hin*dⅢ, pRNAT-U6.1/Neo shRNA1/2/3 and the negative control pRNAT-U6.1/Neo shRNA4 were generated. The recombinants were used to transform into competent *E. coli* DH5α. All the inserted sequences were verified by using restriction enzymes and DNA sequencing. Oligo1, 5′ GATCCCGTATAATCCAGCAGGTCAGCAATTGATATCCGT TGCTGACCTGCTGGATTATATTTTTTCCAAA 3′; Oligo2, 5′ AGCTTTTGGAAAAAATATAATCCAGCAGGTCAGCAACGGATATCAATTGCTGACCTGCTGGATTATACGG 3′; Oligo3, 5′ GATCCCGTCTTTCCAGTTAAGGTTGAGATTGATATCCGTCTCAACCTTAACTGGAAAGATTTTTTCCAAA A 3′; Oligo4, 5′ AGCTTTTGGAAAAAATCTTTCCAGTTAAGGTTGAGACGGATATCAATCTCAACCTTAACTGGAAAGACGG 3′; Oligo5, 5′ GATCCCGTATACTGCTTGACCAAGGAAATTGATATCCGTTTCCTTGGTCAAGCAGTATATTTTTTCCAAA 3′; Oligo6, 5′ AGCTTTTGGAAAAAATATACTGCTTGACCAAGGAAACGGATATCAATTTCCTTGGTCAAGCAGTATACGG 3′; Oligo7, 5′ GATCCCGTTTAATCGAGCAGGTCTGCAATTGATATCCGTTGCAGACCTGCTCGATTAAATTTTTTCCAAA 3′; Oligo8, 5′ AGCTTTTGGAAAAAATTTAATCGAGCAGGTCTGCAACGGATATCAATTGCAGACCTGCTCGATTAAACGG 3′.

1.2 Cell culture and plasmid transfection

The rabbit fibroblasts were cultured in a DMEM/F12 medium supplemented with 10% fetal bovine serum (FBS; Hyclone Co., Salt Lake City, UT, USA) and incubated at 37℃ in a humidified incubator with a 5% CO_2 atmosphere. Twenty-four hours prior to the transfection, the fibroblasts from passages 5 to 7 were seeded in a 6 cm culture dishes, and they were grown overnight to 80%～90% confluence. All the recombinant plasmids were linearized by using *Sca*Ⅰ. We transfected 4 dishes with the recombinants pRNAT-U6.1/Neo shRNA1/2/3 and the negative

control pRNAT-U6.1/Neo shRNA4 by using lipofectamine™ 2000 (Invitrogen) according to the manufacturer's specifications. Six hours after the transfection, the culture medium was replaced with fresh medium.When the cells had reached confluence, the transfected cells were digested with 0.05% trypsin-EDTA, and the digested cells were transferred into three 24-well plates. Forty-eight hours later, a DMEM/F12 medium supplemented with 800 mg/L G418 was used to select the integrated clones.Ten days later, the medium was replaced with a conditioned medium containing 200 mg/L G418. The conditioned medium was obtained by using the procedure described by Li *et al* [16]. The transgenic cell clones were inspected and then digested with 0.05% trypsin-EDTA and transferred to a 24-well plate. The transgenic cells were allowed to reach confluence, following which they were passaged for further experiments or frozen in liquid nitrogen.

1.3 PCR of genomic DNA

The total cellular DNA was extracted from the amplified clones, and the product was used as a PCR template. The following sequences were used as PCR primers for *HGPRT*: 5′ TTCTTGGGTAGTTTGCAG-TT 3′ (sense); 5′ CTATTTAGAAGGCACAGTCG 3′ (antisense). These sequences are located outside the silencing target region of *HGPRT*. The expected size of the target fragment of the vectors was 250 bp. The following conditions were used for the PCR cycles: 95℃ for 4 min, followed by 35 cycles of 94℃ for 30 s, 56℃ for 30 s, 72℃ for 30 s, and a final extension at 72℃ for 7 min. The PCR products were size-fractionated by 1.2% agarose gel electrophoresis. Meanwhile, the target fragments were extracted by using the Gel Extraction Kit and linked with pMD18-T vectors, which were then used for DNA sequencing.

1.4 RT-PCR analysis

The cDNA was synthesized by extracting and reverse transcribing the total cellular RNA by using Cells-to-cDNA™ Ⅱ according to the manufacturer's instructions (Ambion). The *β-actin* gene was selected as the most stably expressed control gene for normalization. PCR was performed with primers for *HGPRT* [5′ AGTGATGATGAACCGGGATA 3′(sense); 5′ AGAGGGCTACAATGTGATGG 3′ (antisense)] and *β-actin* [5′ CACACGGTGCCCATCTACG 3′ (sense); 5′ GCCATCTCCTGCTCGAAGTC 3′ (antisense)]. RT-PCR (ABI PRISM 7300; Perkin Elmer Applied Biosystems, USA) was performed by using the SYBR® Green PCR kit in accordance with the manufacturer's protocol. The PCR conditions consisted of 42 cycles at 95℃ for 15 min, 94℃ for 15 s, 58℃ for 30 s, and 72℃ for 30 s. The standard-curve method was used to determine the relative amounts of RNA transcripts for the genes of interest. In addition, a negative control was established by performing an RT-PCR procedure with all the components except for cDNA.

1.5 Western blotting

The proteins were extracted from transgenic fibroblasts and normal fibroblasts; the protein concentrations were determined using a photometer (Eppendorf, Germany). *β-actin* was used as an internal standard to quantify the *HGPRT* expression in the transfected cells. The protein extract was separated by using 12% SDS-PAGE and transferred to a Hybond polyvinylidene fluoride (PVDF) membrane. The membrane was blocked by using a Tris-buffer saline solution (pH 7.6) containing 0.05% Tween-20 (TBS/T) and 5% milk, and it was incubated with specific antibodies overnight at 4℃. We used goat antihuman HGPRT (Santa Cruz, USA) as the primary antibody; there is a high degree of sequence conservation between rabbits and humans[17]; therefore, we assumed that goat antihuman HGPRT could be used for the detection of rabbit HGPRT. The membrane was incubated with different horseradish peroxidase (HRP)-conjugated secondary antibodies for 1 h at room temperature, and the immunoreactive proteins were visualized with the peroxidase-based ECL Plus detection system (Amersham), according to the manufacturer's instructions.

1.6 Nuclear transfer

Oocyte collection and nuclear transfers were performed as described by Li *et al* [5]. Briefly, adult female New Zealand White rabbits were superovulated, and the ovulated oocytes were recovered by flushing the oviducts with pre-warmed RD medium (50% RMPI 1640 and 50% DMEM) supplemented with 10% FBS and 10 mmol/L Hepes (Hepes balanced RD hRD). The cumulus cells were removed by a 10 min exposure to an hRD medium containing 100 U/ml hyaluronidase (Sigma Chemical Co.) and then pipetted through a suitable pipette. The oocytes were incubated in an hRD medium supplemented with 7.5 mg/L cytochalasin B for 10 min before enucleation. The metaphase plate was

visualized under an inverted microscope equipped with a Spindleview system (Cambridge Research & Instrumentation Inc., Wobum, MA, USA), and the plate, along with a small quantity of cytoplasm, was removed by using a fine needle. The donor cells were inserted into the perivitelline space of the enucleated oocytes. The reconstructed couplets were electrofused by subjecting them to 3 pulses (20 μs; DC, 3.0 kV/cm) using an Electro Cell Manipulator, and incubated at 38℃ in RD medium supplemented with 10% FBS, 2 mmol/L glutanmine, 223 μmol/L sodium pyruvate and 100 μmol/L MEM non-essential amino acids (modified RD, mRD). Eighty minutes after fusion, the fused embryos (NT-embryos) were activated by a second set of identical electropulses, and incubated in mRD containing 5 mg/L cycloheximide and 2 mmol/L 6-dimethylaminopurine (Sigma Chemical Co.) for 1 h at 38℃. After activation, the NT-embryos were incubated in mRD for 2 h, and transferred to 50 μl of upgraded B2 medium (Laboratoire C.C.D, Paris, France) supplemented with 2.5% FBS. A fluorescence microscope was used to detect green fluorescent protein (GFP) expression at each stage.

2 Results

2.1 Identification of shRNA-expression plasmids

The recombinant plasmids contain 2 restriction enzyme sites for *Eco*RⅤ/*Pst* Ⅰ, while the primitive plasmid contains only 1 site for *Pst* Ⅰ. The digestion of the plasmids with *Eco*RⅤ/*Pst* Ⅰ resulted in a 6 380 bp fragment from the primitive plasmid, and 2 661 bp and 3 771 bp fragments from the recombinant plasmids (Figure 1). The results of DNA sequencing provided further confirmation of the presence of the recombinant plasmids, indicating that all the shRNA-expression plasmids carried the appropriate sequence (Data not shown).

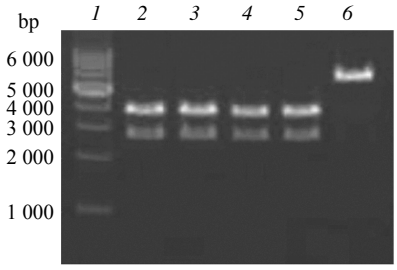

Fig. 1　Identification of shRNA expression plasmids by using restriction endonucleases

The digestion of the plasmids with *Eco*RⅤ/*Pst* Ⅰ resulted in a 6 380 bp fragment from the primitive plasmid, and 2 661 bp and 3 771 bp fragments from the recombinant plasmids. *1*: 1 kb DNA marker; *2*～*5*: pRNAT-U6.1/Neo shRNA1/2/3/4; *6*: pRNAT-U6.1/Neo.

2.2 Generation of stable transfectants

G418 selection was used to obtain stable transfected cell lines from the rabbit fibroblasts (Figure 2a, c); we observed that a few cells aged and lost their growing ability after G418 selection. Well-grown colonies were passaged in a 24-well plate after reaching up to 80% confluence, and then transferred to 35-mm culture dishes to facilitate expansion. The expression of the reporter gene (GFP) was assessed by visualizing the fluorescence (Figure 2b, d).

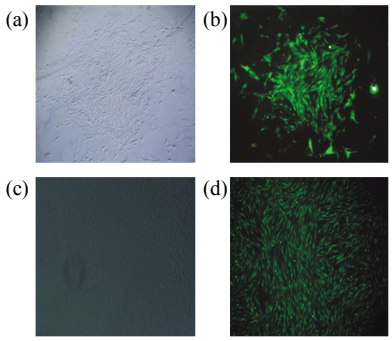

Fig. 2　Expression of GFP in the fibroblasts after G418-selection immediately and after stable passaging

(a, b) Resistant cell clones after the G418 selection. (c, d) Stably amplified cells with GFP expression. (a, c) Bright field microscopic image corresponding to (b) and (d), respectively. Original magnification 40×.

2.3 Identification of transgenic rabbit fibroblast clones by PCR

In order to obtain further confirmation of the insert-mediated transgenesis in the rabbit fibroblasts, we performed a PCR assay of the obtained clones (Figure 3); the target insert was integrated in the genome at a high rate of efficiency (Table 1). We inferred that the transgenes had stably integrated into the genome of the rabbit fibroblasts.

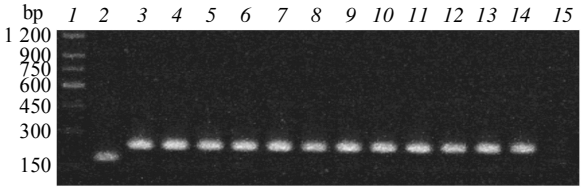

Fig. 3　Identification of transgenic rabbit fibroblast clones by PCR

A specific 250 bp fragment was amplified from all the transgenic rabbit fibroblast clones. *1*: 150 bp DNA marker; *2*: pRNAT-U6.1/Neo; *3*, *7*, and *11*: pRNAT-U6.1/Neo shRNA1/2/3, respectively; *4*～*6*: pRNAT-U6.1/Neo shRNA1 transgenic rabbit fibroblast clones; *8*～*10*: pRNAT-U6.1/Neo shRNA2 transgenic rabbit fibroblast clones; *12*～*14*: pRNAT-U6.1/Neo shRNA3 transgenic rabbit fibroblast clones; *15*: Normal fibroblast.

Table 1 PCR analysis on transgenic fibroblast clones

Type of transgenic fibroblast	Accounts of selectable cell clones	Number of positive cell clones	Number of negative cell clones	Positive rate by PCR analysis/%
shRNA1	7	6	1	85.7
shRNA2	8	6	2	75.0
shRNA3	6	6	0	100.0
shRNA4	9	7	2	77.8
Total	30	25	5	83.3

Positive rate of transgenic cell clones determined by PCR identification; shRNA1/2/3 was used to test the RNAi effect, and shRNA4 was used as a control vector.

2.4 RT-PCR and Western blotting

The results of quantitative RT-PCR demonstrated that the 3 *HGPRT* shRNA vectors caused 46.8%, 68.3%, and 63.5% suppression of HGPRT mRNA expression in comparison with the control, and the pRNAT-U6.1/Neo shRNA2 was found to be most efficient in reducing the *HGPRT* mRNA expression (Figure 4). The results of Western blot analysis revealed that all the 3 *HGPRT* shRNAs efficiently inhibited the expression of *HGPRT* expression, in accordance with the results of RT-PCR analysis (Figure 5).

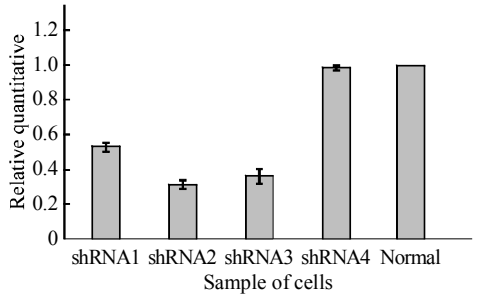

Fig. 4 Relative quantitative PCR using the comparative *C*t method for the detection of *HGPRT* mRNA levels in transgenic fibroblasts

The relative amount of *HGPRT* mRNA was determined by using $2^{-\Delta\Delta Ct}$. *C*t refers to number of cycles required for the intensity of fluorescence to reach the fluorescence threshold; $\Delta\Delta Ct = (C_{tHGPRT}-C_{t\beta\text{-actin}})_{experiment}-(C_{tHGPRT}-C_{t\beta\text{-actin}})_{control}$. shRNA1/2/3 compared with the control ($P < 0.01$). Sample 4 compared with the control ($P > 0.05$). Data represent the average of 3 independent experiments ($n = 3$, paired t test). The bars indicate standard deviation.

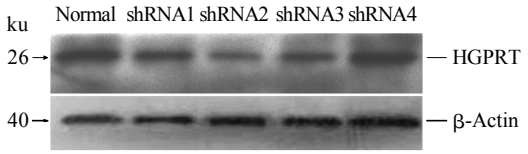

Fig. 5 Western blot analysis of HGPRT protein levels in rabbit fibroblasts after transfection with shRNA expression plasmids

shRNA1/2/3 exhibit different degrees of reduction in HGPRT expression in comparison with that of the normal, and shRNA4 exhibits no obvious difference in comparison with the normal.

2.5 Nuclear transfer

The reconstructed couplets were produced by using normal fibroblasts and transgenic fibroblasts. The performance of the normal and transgenic groups was compared at each preimplantation stage. As shown in Table 2, a higher percentage of NT-embryos in the normal group entered cleavage (normal ∶ transgenic, 87.5% ∶ 79.1%; $P > 0.05$), and a slightly higher percentage of NT-embryos in the normal group developed into blastocysts (normal ∶ transgenic, 32.5% ∶ 27.8%; $P > 0.05$). GFP expression was detected at all the stages (Figure 6).

Table 2 Effect of donor cells on nuclear transfer embryos *in vitro* development

Donor cell lines	NT oocytes	Fused oocytes(%)	2-cell embryos(%)	4-cell embryos(%)	8-cell embryos(%)	16-cell embryos(%)	Blastocyts(%)
shRNA1	108	87(80.6)	72(82.8)	66(75.9)	60(70.0)	49(56.3)	26(29.9)
shRNA2	97	77(79.4)	60(77.9)	53(68.8)	48(62.3)	40(60.0)	22(28.6)
shRNA3	115	95(82.6)	73(76.8)	68(71.6)	57(60.0)	45(47.4)	24(25.3)
shRNA4	101	90(89.1)	71(78.9)	67(74.4)	61(67.8)	50(55.6)	25(27.8)
Total	421	349(82.9)	276(79.1)[a]	254(72.8)	226(64.8)	184(52.7)	97(27.8)[c]
Normal cell	89	80(89.9)	70 (87.5)[b]	65(81.3)	59(73.8)	49(61.3)	26(32.5)[d]

Chi-square analyses demonstrating the statistical differences between a and b ($P > 0.05$), c and d ($P > 0.05$). shRNA1/2/3/4 constituted the transgenic-cell group, and normal cells were used in the control group.

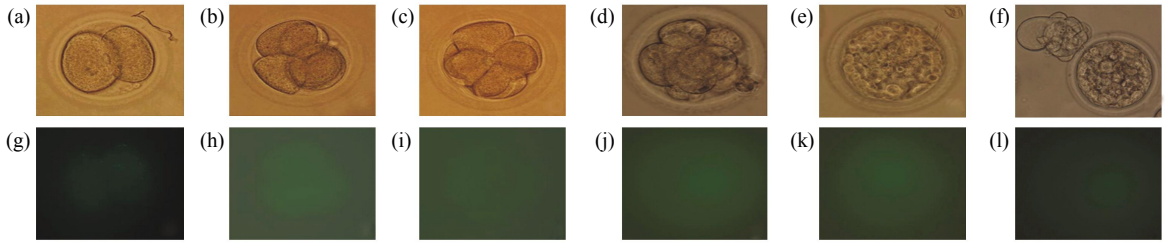

Fig. 6　The development of cloned embryos from the transgenic rabbit fibroblast cells *in vitro*

(a, g) 2-cell NT embryos cultured *in vitro*. (b, h) 4-cell NT embryos cultured *in vitro*. (c, i) 8-cell NT embryos cultured *in vitro*. (d, j) 16-cell NT embryos cultured *in vitro*. (e, k) Blastocysts of NT embryos cultured *in vitro*. (f, l) Hatching of *in vitro*-cultured blastocyst. (g～l) Green fluorescent images showing cGFP expression in NT embryos. (a～f) Bright field microscopic images corresponding to (g～l). Original magnification 400×.

3　Discussion

HR-based gene-knockout or dsRNA-dependent gene-knockdown methods can be used to determine the function of a particular gene or generate animal models for loss-of-function assays of a particular gene. However, the knockout technologies are extremely laborious and time consuming. RNAi provides an easy and inexpensive alternative for the functional analysis of genes and allows researchers to study the dosage effect of a particular gene[18]. Furthermore, it may be possible to develop RNAi transgenic animals by using NT. The inheritance of the recombinant genetic material in the offspring obtained by SCNT is more stable than that in case of microinjection, and the positive rate is higher. In addition, this approach is very useful in studying the issues associated with sexuality.

In this study, we were able to obtain cell lines carrying the stably integrated transgene, as evidenced by PCR identification. Real-time PCR analysis revealed that the 3 shRNAs induced 46.8%, 68.3%, and 63.5% suppression, which was in accordance with the results of Western blotting analysis. These cells were selected and used in SCNT to produce cloned transgenic rabbit embryos, which subsequently developed into blastocysts.

We constructed shRNA-expressing vectors targeting *HGPRT* by using pRNAT-U6.1/Neo, which contains a U6 promoter, an expression cassette for the reporter gene (*cGFP*), and a neomycin-resistance gene. The shRNA expression is driven by the U6 promoter of RNA polymerase Ⅲ. *cGFP* was used to track the transfection and integration efficiency, and a neomycin-resistance gene was used to establish a stable cell line. This experiment proves that this method can induce stable and persistent inhibition of the expression of the target gene in mammalian cells.

In the light of certain adverse effects observed during the *in vitro* cultivation, transfection, and G418-selection of rabbit fibroblasts, we chose to use lipofection-mediated transfection; this method has a better transfection rate and is more convenient to use than the electroporation method [19]. However, we observed that some of the positive clones were not amplified, and the existing cell clones were divided into 2 categories: fibroblast and irregular clones, a phenomenon that can be attributed to the negative effects of the G418 selection and high passaging. The negative cell clones of PCR identification could be the result of a failure in integration or a loss of exogenous gene during selection. These problems need to be investigated in further studies. In general, we found that the use of the conditioned medium and fibroblasts up to the sixth generation were helpful in the acquirement of stable transfected cell lines. The non-transgenic cells can be eliminated by exposing them to low-dosage G418 during the phases of expansion.

The transgenic cell lines were used as donors for SCNT, and the NT-embryos were cultured *in vitro* to various stages of preimplantation development. The blastocyst development rate was 27.8%, which was slightly lower than that of normal group. The possible explanations are cell degeneration during transfection or genome disturbances arising from integration of exogenous genes. Therefore, long-term passage and drug selection are not beneficial to embryo development, in accordance with previous studies[20, 21]. GFP expression was detected at every embryonic

stage, and the intensity of GFP expression was found to increase with the development of the NT-embryo, a phenomenon that may be attributed to the increase in cell numbers or the gradual accumulation of *c*GFP.

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应用 RNA 干扰技术体外抑制兔成纤维细胞 *HGPRT* 的表达及其核移植研究 *

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摘要　次黄嘌呤 - 鸟嘌呤磷酸核糖基转移酶(hypoxanthine guanine phosphoribosyltransferase，HGPRT)的功能缺失与痛风、肾结石和雷纳综合症(Lesch-Nyhan Syndrome)等疾病相关．制作 HGPRT 基因表达降低的模式动物，将有利于人们对这种疾病的发病机理和治疗做进一步的研究．构建了针对 HGPRT 基因表达的 shRNA 干扰载体，并将质粒转染兔成纤维细胞，获得携带该干扰片段的转基因细胞系，经 PCR 鉴定转基因成纤维细胞克隆阳性率为 83.3%．RT-PCR 及 Western blot 检测结果表明转基因干扰成纤维细胞系 HGPRT mRNA 和蛋白质表达量明显降低．最后，以转基因成纤维细胞进行核移植，囊胚率为 27.8%，与正常来源的成纤维细胞囊胚率相比较差异不显著．说明，通过 RNAi 可稳定干扰兔成纤维细胞 *HGPRT* 基因的表达，为进一步通过核移植技术建立 *HGPRT* RNAi 转基因兔模型创造条件．

关键词　RNA 干扰，兔成纤维体细胞，次黄嘌呤 - 鸟嘌呤磷酸核糖基转移酶(HGPRT)，核移植
学科分类号　Q7　　**DOI:** 10.3724/SP.J.1206.2008.00731

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* 上海市科委基础研究重点项目(08JC1413900), 上海市科技兴农重点攻关项目(沪农科攻字(2006)第 5-3 号)和上海市重点学科建设项目(S30201).
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收稿日期：2008-10-23，接受日期：2009-01-09