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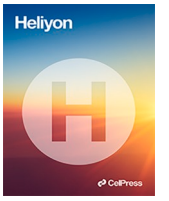

Research article

Genetically engineered mouse model of HPV16 E6-E7 with vaginal-cervical intraepithelial neoplasia and decreased immunity

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ABSTRACT

Objective: To construct models of high-risk human papillomavirus (HPV) infection with precancerous lesions or cervical cancer and explore the immune function.
Methods: Using CRISPR/Cas9, the expression vector *HPV16-E6-E7*-Rosa26 was microinjected into fertilized eggs of C57BL/6 N mice using homologous recombination, and the F0 generation was obtained for reproduction. Then, the formation of precancerous lesions was promoted via intramuscular injection of estradiol. Presence of precancerous cervical-vaginal intraepithelial lesions, Ki67 and p16 expression levels, and $CD8^+$ T cell proportions in the spleen were evaluated.
Results: Two F0 generation mice exhibited correct the homologous recombination. Seven positive mice were identified in the F1 generation. After breeding and mating, 25 homozygous and 11 heterozygous *HPV16-E6-E7*-engineered mice were obtained from the F2 generation. After estradiol benzoate treatment, the cervical-vaginal epithelium appeared as precancerous lesions with positive Ki67 and p16 expression. The percentage of $CD8^+$ T cells decreased.
Conclusion: HPV16-E6-E7-Rosa26 induced low immune function in mice, and provides a good model for the basic research of the mechanisms of action of HPV infection-associated precancerous lesions or cervical cancer.

1. Background and introduction

Cervical cancer is predominantly caused by high-risk human papillomavirus (HPV), and develops as a series of precancerous lesions over several years. Among HPV types, HPV16 and 18 together account for approximately 75 % of all cervical cancer cases [1]. Notably, more than half of the cervical cancer cases are attributed to HPV16 persistent infection [2]. HPV viral genome encodes two major oncogenes, *E6* and *E7*, which not only play crucial roles in carcinogenesis [3], but also contribute significantly to the immunosuppressive microenvironment and immune evasion mechanisms of cervical cancer [4]. Therefore, investigating the molecular mechanisms underlying the actions of E6/E7 holds significant clinical implications. However, a lack of an ideal animal model that accurately

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recapitulates HPV infection-associated precancerous lesions or cervical cancer has been a major obstacle hindering both clinical and basic research on this topic. In 1994. the first transgenic mouse model with progressive squamous epithelial neoplasia expressing human papillomavirus type 16 was constructed as keratin-14(K14) promoter [5]. These transgenic mice have been extensively utilized over several years. Plasmids containing Open Reading Frame(ORFs) were constructed, and the entire early coding region of HPV16 was excised from these plasmids and cloned into the BamHI site of the keratin-14(K14)-expressing plasmid to generate plasmids pK14–1203, pK14-16Nt, and pK14-l6Pt. Fragment concentrations were adjusted for microinjection. Transgenic animals were generated using standard techniques by microinjecting B6D2/F2 embryos and backcrosses to create transgenic lines. The animals predominantly used were C57BL/6 and BALB/c mice and the lineages were maintained as heterozygotes. It should be noted that this model is hormone-dependent; chronic estradiol treatment at doses ranging from 0.1 to 0.25 mg/90-d can induce invasive squamous cancers in vulva, vagina, and outer cervix regions in K14-HPV16 mice [6,7].

To date, despite recent advancements in techniques, the utilization of this transgenic mouse model persists in recent years. A novel Gt(ROSA)26S or targeted transgene has been developed based on CRISPR-Cas9, that allows a gene knock-in (KI) or precise control over the order and timing of multiple genetic mutations in mice [8]. Consequently, it may be feasible to obtain transgenic animals that specifically and stably express HPV16 E6/E7 in the cervico-vagina for further investigations into mechanisms of action, drug evaluation, and vaccine development. At present, a variety of HPV16 transgenic mice expressing different components of HPV16 have been developed successively [9–11], and the adoption of HPV16 E6/E7 transgenic mice has been confirmed to induce strong T cell immune tolerance [12,13]. However, existing models possess limitations such as complex preparation technology, breeding challenges, poor genetic stability, and unstable expression of target proteins, which do not meet the requirements for further study. Currently, assessment of the disease immune status in *HPV16 E6/E7* transgenic mouse models of associated HPV infection and precancerous lesions are lacking.

In the present study, we adopted CRISPR/Cas9 technology, which has been described previously [14,15] using the human keratin 14 promoter (K14 promoter). We aimed to create a PiggyBac transgenic line expressing HPV16-E6-E7 under control of the K14 promoter in C57BL/6 N mice. The K14 promoter is a tissue-specific promoter for the PiggyBac vector, and the "K14 promoter-Kozak-HPV16-E6-P2A-HPV16-E7-rBG pA" cassette is flanked by two PiggyBac inverted terminal repeats (ITRs) to facilitate transposon-mediated transgene integration. Through homologous recombination, HPV16-E6-E7 expression frames were inserted into ROSA26 gene loci to obtain Hpv16-e6-e7-Rosa26-expressing recombinant vectors containing *HPV16-E6-E7*. CRISPR/Cas9 technology and the K14 promoter, which is connected to the HPV early reading frame, were used to enable high-level expression of E6/E7 in the epithelium by microinjection [9]. The PiggyBac vector was then co-injected with a transposon into fertilized eggs from C57BL/6 N mice. Pups were genotyped by polymerase chain reaction (PCR)to identify those carrying the desired PiggyBac transgene. Finally, positive founder mice were counter-screened for transposons. Simultaneously, gene editing was performed in C57BL/6 N mice by microinjection to reduce the immunogenicity of HPV16-E6-E7. Finally, two homologous recombinant F0 generation mice were obtained. The F0 generation of mice was then mated with wild-type mice to obtain the F1 generation, followed by the F2 generation. In this study, immune and cervical intraepithelial neoplasia (CIN) were observed in transgenic mice treated for 3 months. Therefore, the present study provides a suitable experimental animal model.

2. Materials and methods

2.1. Experimental animals and materials

In this experiment, SPF C57BL/6 N mice (purchased from Shanghai Nanfang Model Biotechnology Co., LTD.) were utilized and strict adherence to animal welfare and ethics regulations. The plasmid of gRNA and Cas9 were constructed by Cyagen Biosciences Inc. (China). Primer synthesis services were provided by Borui-Bio Technology Co.(Shanghai, China.). Other materials used included as follows: DNA extraction kit (Cwbio, Technology Co., Jiangsu, China.), PE anti-mouse CD3 antibody (100308, Armenian Hamster lgG), CD8a (100722; BioLegend), red cell lysis solution (C3702; Beyotime), type I collagenase (Sigma, USA), DNase I (Sigma, USA), Ki67 rabbit polyclonal antibody (TA336650; OriGene), P16 rabbit monoclonal antibody (TA592012; OriGene).

2.2. Construction and identification of the vector and CRISPR/Cas9 plasmid

Conventional methods were used to clone human papillomavirus *HPV16-E6-E7*. Initially, amplification of *HPV16 E6/E7* was performed. Subsequently, the glue was recovered and connected to the construction vector K14 promotor-Kozak-HPV16-E6-P2A-HPV16-E7-rBGpA. Next, CRISPR/Cas9 knock-in technology was used. The sgRNA targeted sequences were as follows: gRNA1 was GGCAGGCTTAAAGGCTAACC-TGG, and the sgRNA2 was CTCCAGTCTTTCTAGAAGAT-GGG. In this way, specific *HPV16-E6-E7* foreign genes were introduced through Cas9 nuclease cleavage and homologous recombination of the homologous arms. The target vector was constructed using In-Fusion technology. The PCR product and plasmid were digested with ApaLI + NheI for verification.

2.3. PCR identification of genetically engineered mice

The primers used in this study were as follows: Primers1: Product length: 451 bp; F1:5′- GCTCTGAGTTGTTATCAGTAAGGGA - 3'; R1: 5′- TTATGCAGGGATCAGGAATAGAAGC - 3'; Primers2: Product length: 4089 bp; F2:5′GCTCTGAGTTGTTATCAGTAAGGGA-3'; R2: 5′-ATACTCCGAGGCGGATCACAA-3'. Rat tail lysis solution was prepared and digestion was performed prior to amplification using a PCR hybrid system. Agarose gel electrophoresis was performed to detect gradient PCR amplification products. Finally, a gel imaging

system was used for observation and analysis.

2.4. Tumor promotion program in HPV16-E6-E7-engineered mice

Three transgenic mice, aged 6 weeks, were selected and injected with 0.2 mg estradiol benzoate in the abdomen, every other day regularly for 3 months. The *HPV16-E6-E7*-treated female mice (n = 3 ♀) were used as the experimental group and untreated transgenic mice (n = 3 ♀) or wild-type C57 mice ((n = 3 ♀) as the control groups. Subcutaneous observations were made on spleen, cervical, and vaginal tissues.

2.5. Flow **cytometry** analysis

Mice were treated with estrogen for 3 months and euthanized using CO_2. Subsequently, the spleens were excised and cut into fragments measuring 1–2 mm in size, which were then digested with type I collagenase. The resulting samples were placed in RPMI 1640 medium and incubated at 37 °C for 35 min with DNase I (60 U/mL). Following digestion, the cell suspension was filtered through a 70 μm cell filter, and red cell lysis solution was added according to the manufacturer's instructions. Finally, flow cytometry analysis was performed to determine the proportions of CD3 and CD8 cells in mouse spleens using PE anti-mouse CD3 antibody (1:150) and PE/cy7 anti-mouse CD8a antibody (1:50).

2.6. **Hematoxylin** and eosin and immunohistochemical staining and analysis

After obtaining fresh tissue samples and performing fixation, the conventional method of paraffin embedding was employed. Hematoxylin and eosin as well as immunohistochemical staining were conducted following a standardized protocol. Primary antibodies against Ki67 or p16 were used at a dilution ratio of (1:100). Subsequently, pika Universal secondary antibody was added after incubation with the primary antibody. For each section, five high-power visual fields were randomly selected and the two proteins were mainly expressed at the nuclear site.

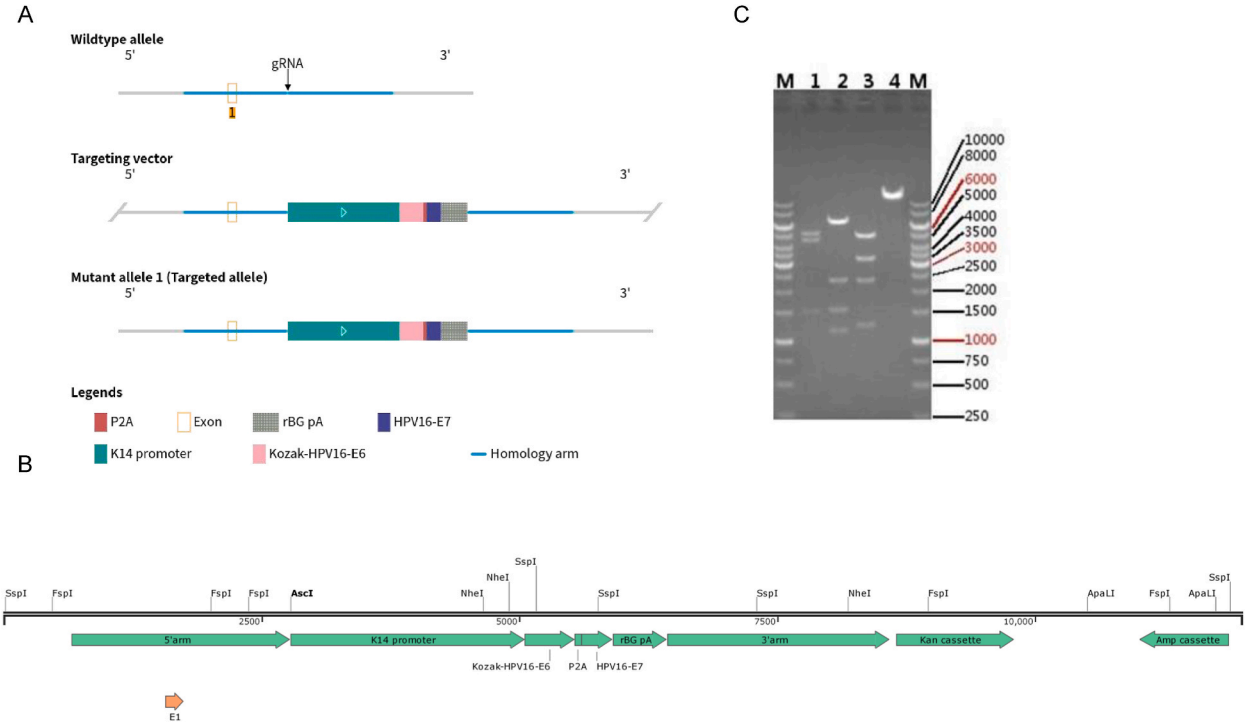


Fig. 1. Sketch map of the HPV16-E6-E7-ROSA26 vector and detection using enzyme digestion A, Design of the recombinant HPV16-E6-E7-ROSA26 vector, including a sketch map of the wild-type allele (gRNA vector) and targeting vector, as well as the mutant allele 1 (targeted allele). B, Restriction site map of the vector. C, Electrophoresis results demonstrating target fragment size. The vector was cleaved using different enzymes, and the accuracy of vector construction was determined according to fragment size after cleavage. Lane 1. Five fragments (5.1, 4.6, 1.5, 0.6, and 0.1 kb) were cleaved using SspI. Lane 2. Five fragments (6.6, 2.3, 1.5, 1.2, and 0.4 kb) were cleaved using FspI. Lane 3. Five fragments (4.9, 3.3, 2.3, 1.2, and 0.3 kb) were cleaved using ApaLI and NheI. Lane 4. Five fragments of 12 kb each were cleaved using AscI.

2.7. Statistical analysis

Statistical analyses were conducted using an unpaired two-tailed Student's *t*-test with GraphPad Prism 6 software (GraphPad Software, Inc., San Diego, CA, USA). The level of statistical significance was set at $P < 0.05$.

3. Results

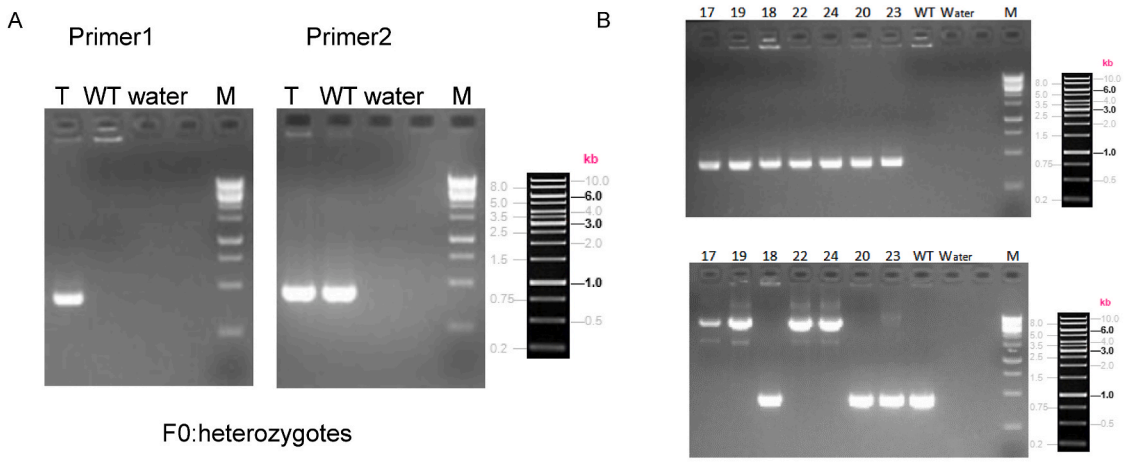
3.1. Construction of the HPV16-E6-E7-ROSA26 vector and detection using enzyme digestion

Firstly, the design of target and expression vectors was carried out, encompassing the wild-type allele (gRNA vector) and targeting vector, along with mutant allele 1 (targeted allele). The recombinant HPV16-E6-E7-ROSA26 vector is depicted in Fig. 1A. Corresponding single guideRNAs (sgRNA) for target genes were designed and constructed, ensuring accurate insertion of Rosa26 without affecting the expression of other genes. Subsequently, the HPV16-E6-E7 expression frame was inserted into the *Rosa26* site. A recombinant vector containing *HPV16-E6-E7* (K14 promoter-Kozak-HPV16-E6-P2A-HPV16-E7-rBG pA) was obtained and confirmed through PCR and electrophoresis analysis.

The enzyme site within the vector is illustrated in Fig. 1B. Electrophoresis results demonstrated that the size of the target fragment corresponded to its theoretical band size (Fig. 1C), confirming precise construction of the recombinant vector harboring K14 promoter and *HPV16 E6/E7*.

3.2. Construction and identification of HPV16-E6-E7-engineered mice

The construction procedure was as follows. The insertion of sgRNA to mouse *ROSA26* was followed by co-injecting the donor vector containing the "K14 promoter-Kozak-HPV16-E6-P2A-HPV16-E7-rBG pA" cassette and Cas9 mRNA into fertilized mouse eggs to generate targeted knock-in offspring. The F0 founder animals were identified by PCR, followed by sequence analysis, and were bred with wild-type mice to test germline transmission and F1 animal generation. Intercross-heterozygous mice were used to generate homozygous mice. In this study, Cas9 mRNA, sgRNA, and recombinant targeting vectors were injected into fertilized eggs of C57BL/6 N mice using a microinjection technique. The fertilized eggs were then transplanted into the fallopian tubes of C57BL/6 N female surrogate mice. We injected 300 fertilized eggs and transplanted them into 10 surrogate mice. F0 mice were identified using PCR after birth. We obtained two positive F0 mice (two heterozygous F0 generation mice).



Identification number	Identification area	Primer name	Primer sequence 5'- 3'	Product size bp MT	Product size bp WT	postscript
1	BJ	A2444-NKIM0046-F5	GCTCTGAGTTGTTATCAGTAAGGGA	451	/	Homozygous: one band with 451 bp Heterozygous: two bands with 451 bp and 434 bp WT: one band with 434 bp
		A2445-NKIM0046-R21	TTATGCAGGGATCAGGAATAGAAGC			
2	WT	Rosa26-CX-F	GCTCTGAGTTGTTATCAGTAAGGGA	4089	434	
		Rosa26-CX-R	ATACTCCGAGGCGGATCACAA			

Fig. 2. Identification of F0/F1 *HPV16-E6-E7*-engineered mice A, Heterozygous F0 generation mice. Primer 1 product size, 451 bp; Primer 2 product size, 434 bp; heterozygous, two bands of 451 and 434 bp; wild type (WT), one band of 434 bp; water (control), no band. B, F1 generation mice carrying *HPV16-E6-E7* were analyzed using PCR. Product size, 4089 bp; WT allele, 434 bp; homozygous, one band of 451 bp; heterozygous, two bands of 451 and 434 bp; WT, one band of 434 bp. The results showed that the F1 generation mice numbered 17, 18, 19, 20, 22, 23, and 24 were positive (17, 19, 22, 24 were homozygous, whereas 18, 20, and 23 were heterozygous). Homozygote, one band of 451 bp; heterozygote, two bands of 451 and 434 bp; WT, one band of 434 bp.

The PCR results demonstrated successful homologous recombination of *HPV16-E6-E7* in the positive F0 representative mice (Fig. 2A). F0 generation mice were mated with wild-type C57BL/6 N mice to generate F1 offspring, which were subsequently bred and mated with each other. PCR genotype identification was performed on the offspring until four homozygous transgenic offspring mice and three heterozygous engineered offspring mice were obtained (Fig. 2B).

3.3. Breeding and identification of HPV16-E6-E7-engineered mice

F2 generation mice were obtained by selecting F1 generation mice from the same F0 generation with the identical knock-in site and conducting sibling mating after reaching sexual maturity. Theoretically, 25 % of the F2 generation mice exhibited homozygosity for the knock-in site, while 50 % displayed heterozygosity for only one chromosome knock-in, and the remaining 25 % were wild type. Genotypes (homozygous, heterozygous, and wild type) were also identified by PCR. F1 transgenic mice carrying *HPV16-E6-E7* were backcrossed, gradually bred, and purified until homozygous progeny transgenic mice were obtained (Fig. 3A–D). Homozygotes were represented by a band of 451 bp; heterozygotes had two bands of 451 and 434 bp, and the wild type had a band of 434 bp. The *HPV16-E6-E7*-engineered mice of the F2 generation, included 25 homozygous and 11 heterozygous mice. Additionally, Sanger sequencing was performed to confirm correct insertion of *HPV16-E6-E7* in the F2 generation mice (Supplementary File S1).

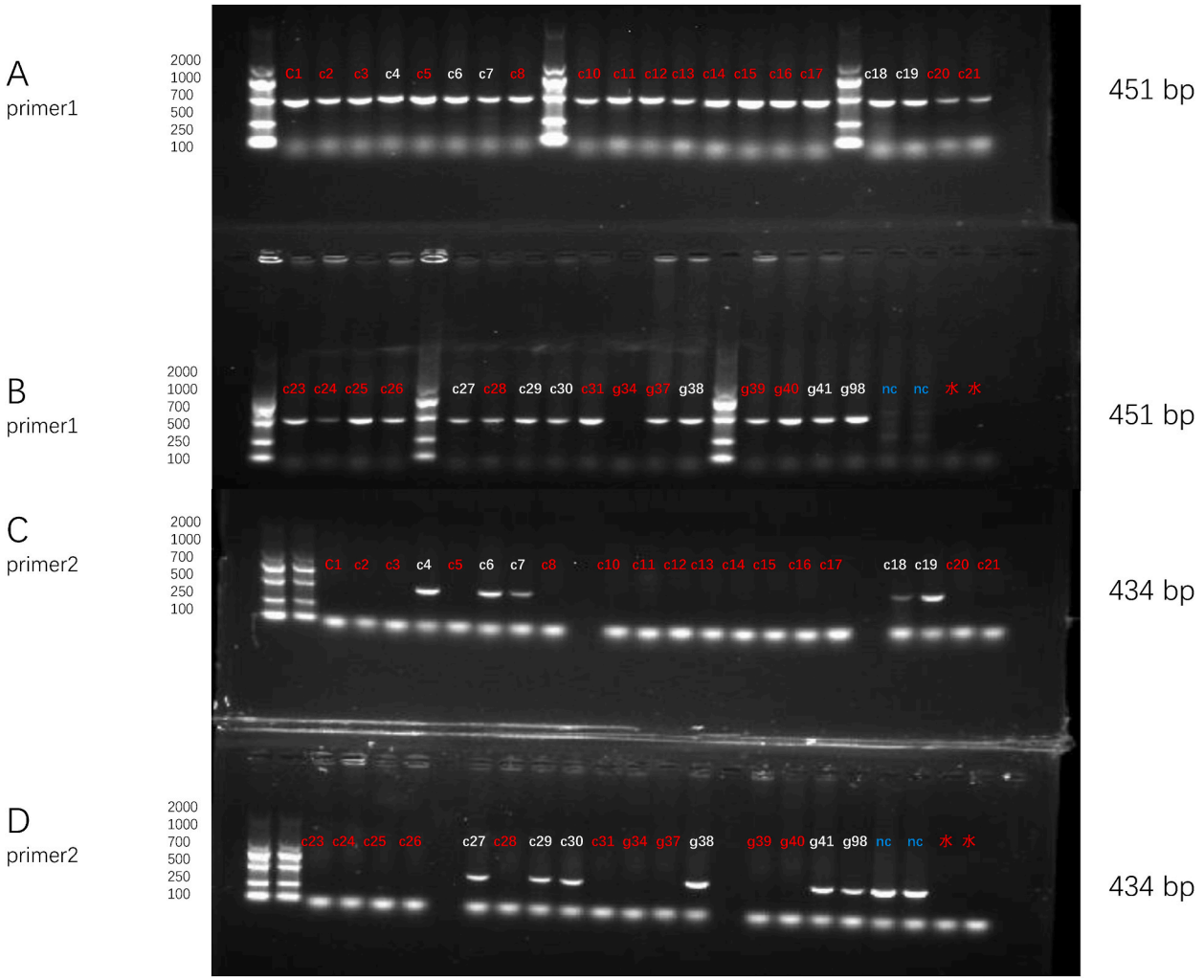


Fig. 3. Identification of the F2 *HPV16-E6-E7*-engineered mice A total of 25 (+/+) and 11 (±) HPV16-E6-E7-engineered mice of the F2 generation were obtained. The results showed that c1, c2, c3, c5, c8, c10, c11, c12, c13, c14, c15, c16, c17, c20, c21, c23, c24, c25, c26, c28, c31, g34, g37, g39, and g40 were homozygotes, whereas c4, c6, c7, c18, c19, c27, c29, c30, g38, g41, and g98 were heterozygotes. Only one band was wild-type at 434 bp. Homozygote, one band of 451 bp; heterozygote, two bands 451 and 434 bp; wild type, one band of 434 bp.

3.4. Intraepithelial neoplasia in cervical-vaginal tissue

To further observe the effect of knock-in HPV16-E6-E7, we assessed the progressive changes in the skin, hair, vulva, and anus of *HPV16-E6-E7* transgenic mice over time (Supplementary Fig. S1). As estrogen has been shown to induce cancer in mice with HPV infection [6], we administered estrogen injections to *HPV16-E6-E7*-engineered mice. After three months, the cervical-vaginal tissue appeared distinctly thickened (Fig. 4A), and a hematoxylin and eosin–stained section exhibited a low-grade squamous intraepithelial lesion (CIN I), and a partial high-grade squamous intraepithelial lesion, which was CIN II (Fig. 4B). Immunohistochemistry results revealed that the expression of Ki67 and p16 in the area of the intraepithelial neoplasia was higher than that in the control group (Fig. 4C and D). In addition, we observed estrogen receptor (ER)expression in both *HPV16-E6-E7* transgenic mice with or without estrogen treatment, as well as in the wild-type mice. The immunohistochemistry results demonstrated that ER was strongly positive in the engineered mice treated with estrogen when compared to that in the engineered mice without estrogen treatment or in the wild-type mice (Fig. 5A-D). Thus, estrogen can promote the formation of intraepithelial neoplasia, even in cancer, if the time is more than 6–8 months [6].

3.5. Immunosuppressive phenomenon might be induced in the engineered mice

The function of $CD8^+$ T cells is associated with immunosuppression and cancer development. Herein, the expression levels of $CD3^+$ and $CD8^+$ T cells in the spleen were compared between engineered mice treated with estrogen and the control group (wild-type C57BL/6 N mice). The percentages of $CD8^+$ T cells in the experimental group were 1.73 %, 4.04 %, and 6.83 %, respectively, of the total cells and the $CD3^+$ T cells accounted for 2.23 %, 4.51 %, and 8.12 %, respectively. In the control wide type mice, $CD8^+$ T cells accounted for 19 %, 14.7 %, and 19.4 %, respectively, of the total cells and $CD3^+$ T cells accounted for 27.5 %, 22.2 %, and 27.7 %, respectively. The differences between the groups were significant ($P < 0.01$, Fig. 6), indicating that an immunosuppressive phenomenon might be induced in this mouse model when CIN have been occurrence.

4. Discussion

HPV infections are associated with the development of cancer, and HPV16 is the most prevalent high-risk HPV type. The viral oncoproteins E6 and E7 have been implicated in the pathogenesis of cervical cancer. However, the details or key factors involved in immune escape and methods to address high-grade squamous intraepithelial lesion or invasive cancer remain elusive. Therefore, constructing transgenic animals that can specifically and consistently express *HPV16-E6-E7* in the cervix holds significant clinical value. In recent years, CRISPR/Cas9 technology has emerged as a powerful tool for gene editing and has revolutionized cell therapy approaches [16–18]. In this study, as depicted in Figs. 1 and 2, compared with traditional technology, *CRISPR/Cas9* technology exhibited good advantages in constructing transgenic animals. The constructed *HPV16-E6-E7* transgenic mice have been bred easily thus far and their genetic characteristics are stable, as shown in Fig. 3. Moreover, our findings indicate that only two oncogenes E6 and

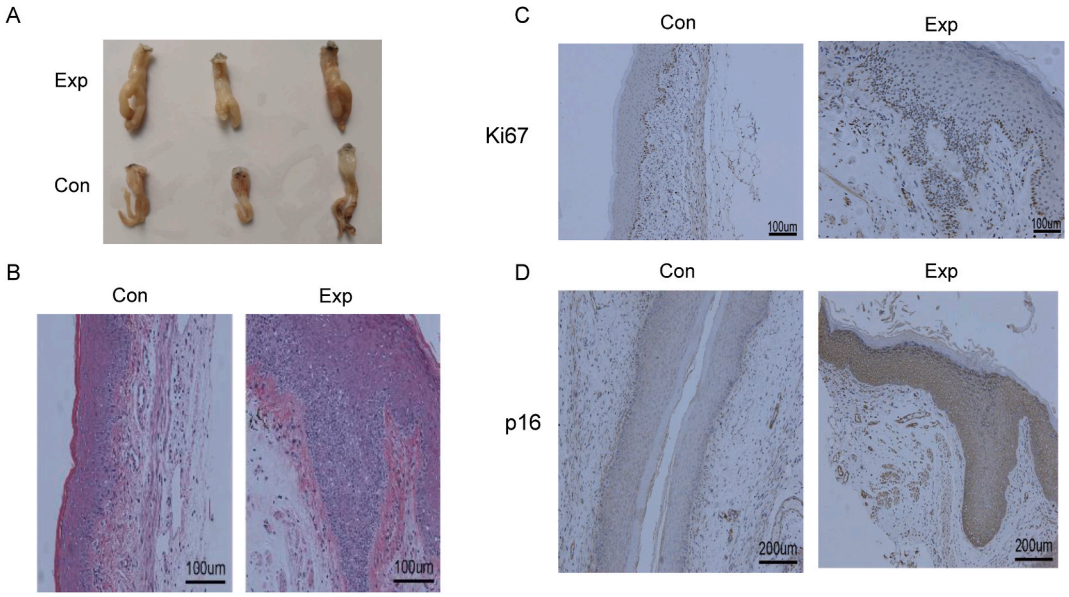


Fig. 4. Intraepithelial neoplasia was promoted in the engineered mice treated with estrogen A, Image of cervical-vaginal tissue of the experimental group (*HPV16-E6-E7*-engineered mice treated with estrogen) and control group (wild-type C57BL/6 N mice). B, Pathological section exhibiting hyperplasia in the experimental group. C and D, Immunohistochemistry showed positive expression of Ki67 and p16 in the experimental group. Scale bar: 100 μm or 200 μm.

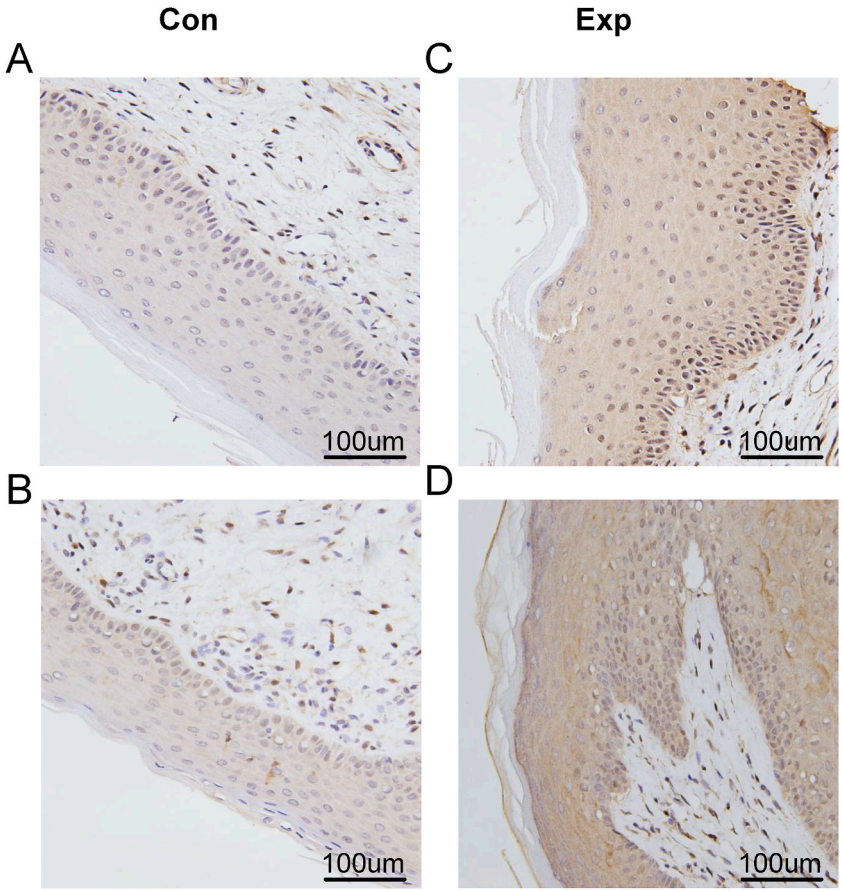


Fig. 5. Expression levels of ER in the transgene mice A-B, ER expression was detected by immunohistochemistry in control (wild-type C57BL/6 N mice) groups. C-D, ER expression was detected by immunohistochemistry in the experimental group. Scale bar: 100 μm.

E7 are sufficient to induce low-grade or high-grade squamous intraepithelial lesions following three months of estrogen treatment, as shown in Fig. 4. This mouse model successfully recapitulates key aspects of HPV-induced carcinogenesis involving E6 and E7 expression. Another reported case of invasive cancer occurred after more than 6–8 months [19]. However, it did not achieve severe lesions within a short period in the present study. In addition, E6 and E7 mouse models can provide more specific data regarding the mechanisms of action the two oncogenes in vivo. *HPV16-E6-E7* mice might have advantages and superiority over HPV16 mice because of their stable generation and convenience. There have been a few reports on immune escape in animal models in the cervical cancer; therefore, this new strain of *HPV16-E6-E7* transgenic mice may need further application. As previously mentioned, E6 and E7 are constitutively expressed as major transforming proteins in precancerous and advanced lesions, making them ideal targets for HPV-induced malignant tumor immunotherapy [13,20,21]. E6 and E7 expression is also associated with several telomere pathways. Tangentially, with increased cell presence, *E6* and *E7* are highly capable of hTERT regulation, ultimately promoting telomere maintenance [22,23]. This process is essential for cancer maintenance because telomeres are shortened with repeated gene replication and cell division [24]. The higher the telomerase activity, the less susceptible the tumor cells are to shortened telomere-related DNA damage [25]. Our study found out that E6 and E7 expression may affect the telomere pathway and cause intraepithelial neoplasia I-II. The HPV16-E6-E7 transgenic mouse is an ideal model for research on HPV infection-associated precancerous lesions and cervical cancer. Moreover, $CD8^+$ T cells have been identified as effector cells in the T cell depletion test [4]; therefore, we also tested T cells in the spleens of the genetically engineered mice in the experimental and control wild-type C57BL/6 N mice groups. Estrogen does not induce immune defects. The results showed distinct T cell depletion in *HPV16-E6/E7* mice treated with estrogen. In the experimental group, $CD8^+$ and $CD3^+$ T cells were significantly reduced compared to those in the control group, which is consistent with the literature. It suggests that the model is highly suitable for studying precancerous cervical lesions.

Therefore, the development of novel *HPV16-E6-E7* transgenic mice would be invaluable for investigating the underlying mechanisms and assessing the efficacy of drugs/vaccines targeting *HPV16-E6-E7* as a therapeutic intervention. In conclusion, the model is especially suitable for an in-depth study and the effect to the immune microenvironment. Further studies are needed to confirm this hypothesis.

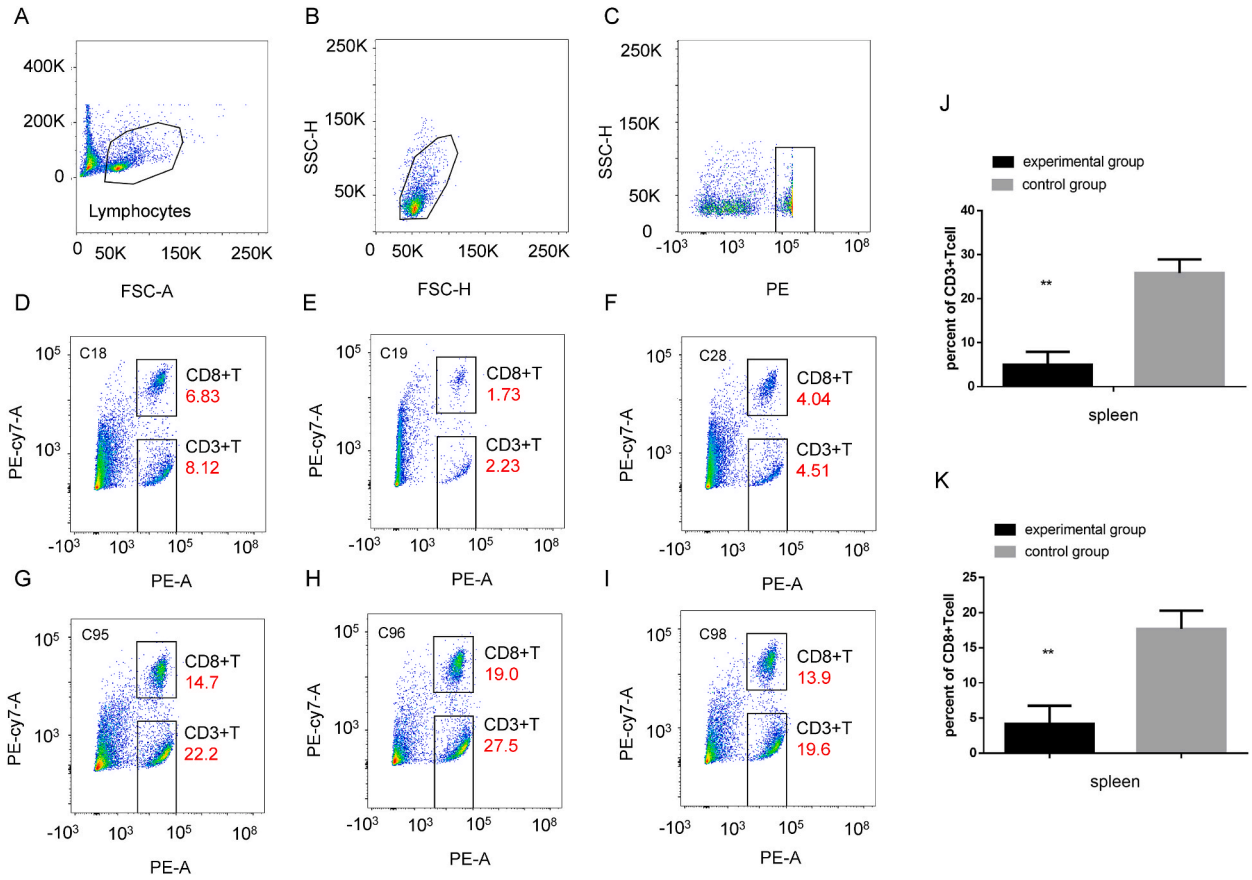


Fig. 6. Expression of CD3$^+$ and CD8$^+$ T cells in the spleen of the experimental group A–C: Flow cytometry procedure of separating CD3$^+$ and CD8$^+$ T cells. D–F: Experimental group; CD8$^+$ T cells ratio accounted for 1.73 %, 4.04 %, and 6.83 % of the total T cells in three mice's spleen respectively, whereas CD3$^+$ T cells accounted for 2.23 %, 4.51 %, and 8.12 % in the mouse spleens. G–I: Control group; CD8$^+$ T cells accounted for 19 %, 14.7 %, and 19.4 % of the total T cells and CD3$^+$ T cells accounted for 27.5 %, 22.2 %, and 27.7 %. J–K: The proportions of CD3$^+$ and CD8$^+$ T cells in the experimental group were significantly higher than those in the control group. ** $P < 0.01$.

5. Conclusion

HPV16-E6-E7-ROSA26 genetically engineered mice obtained using the CRISPR/Cas9 technique exhibited low immune function, and could serve as a good model for HPV infection-associated precancerous lesions or cervical cancer.

6. Limitations

The study's limitations encompass several key aspects. Firstly, by the 1996 PNAS paper, estradiol-treated K14 HPV16 mice developed in situ and invasive carcinoma at 6 months. In present study, all mice were sacrificed at 3 months. That being said, we may need to look at the phenotype of transgenic mice with 6-month treatment. Furthermore, the experimental group includes HPV16-E6-E7 mice with estradiol treatment and the control group is with untreated transgenic mice or wild-type C57 mice. Here, an important control group is missing - which is wild-type C57 mice with estradiol treatment, although we had known that estradiol treatment did not affect the immune system from the previous literature. This needs to be included and verified clearly. Overall, the study offers valuable insights into mice model development, but further research is crucial for advancements.

Ethics statement

The study had approved by the Laboratory welfare and ethics committee of the Army Medical University and we confirm that the experiments complied with all regulations of the animal welfare. The ethical approval number is AMUWE20226267.

Funding

This study was approved by the National Key Technology R&D Program of China, with the approval number: [2019YFC1005200

and 2019YFC1005202].

Data availability statement

The data used during the current study are available from the corresponding author on reasonable request.

Data associated with our study (HPV16-E6-E7-ROSA26 genetically engineered mice) has been deposited into a publicly available repository

https://www.ncbi.nlm.nih.gov/WebSub/

Gene bank Submission ID # 2803040.

CRediT authorship contribution statement

Du Xiurong: Writing – original draft, Investigation, Formal analysis. **Zhou Xin:** Methodology, Formal analysis. **Yang Neng:** Methodology, Investigation. **Deng Li:** Methodology, Investigation, Data curation. **Wang Yanzhou:** Supervision, Project administration, Formal analysis. **Ling Kaijian:** Writing – review & editing, Supervision, Project administration, Data curation, Conceptualization. **Liang Zhiqing:** Supervision, Project administration, Funding acquisition, Conceptualization.

Declaration of generative AI and AI-assisted technologies in the writing process

There is none.

Declaration of competing interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: Zhiqing Liang reports financial support was provided by National Key Technology R&D Program of China. Du xiurong reports a relationship with National Key Technology R&D Program of China that includes: Ling kaijian has patent pending to The Army Medical University. None If there are other authors, they declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

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