



The Expression of Recombinant Human Serum Albumin in the Mammary Gland of Transgenic Mice

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Abstract

Human serum albumin (HSA) is widely used in the clinic for the treatment of several diseases in large amount each year. With the increasing demands of HSA in clinic and limited blood resource, recombinant HSA (rHSA) is becoming an attractive and alternative source for HSA production. In this study, we aimed to express rHSA in the mammary glands of transgenic mice by using a tissue-specific promoter and other regulatory elements. An rHSA expression vector was constructed bearing the cDNA and first intron of HSA under the control of bovine α_{s1} -casein promoter with a 2 × chicken β -globin insulator in the front. Transgenic mice were generated and reverse transcription polymerase chain reaction showed that rHSA was expressed only in the mammary gland, indicating the tissue specificity of the bovine α_{s1} -casein promoter in directing transgene transcription in transgenic mice. Enzyme-linked immunosorbent assay test showed that rHSA was successfully secreted into the milk of transgenic mice with the highest level at 1.98 ± 0.12 g/L. Our results indicate the ability of the bovine α_{s1} -casein promoter to induce successful expression of rHSA in the mammary gland of transgenic mice.

Keywords

- transgenic mice
- mammary gland
- recombinant human serum albumin

Introduction

As the most abundant protein in human plasma, human serum albumin (HSA) has a single nonglycosylated polypeptide chain composed of 585 amino acids with a molecular weight of 66.5 kDa.¹ HSA is widely used in the clinic for the treatment of several diseases including shock, hemodialysis, surgical blood loss, acute respiratory distress syndrome, acute liver failure, and hypoalbuminemia.² Moreover, HSA is used in various biotechnological applications such as fusion proteins, ligand trapping, surgical adhesives, and sealants.^{3–5} Clinically used HSA is normally derived from human plasma; therefore, there is a risk of infection from blood contamination such as human immunodeficiency virus and hepatitis viruses. As a result, recombinant HSA (rHSA) is considered as an alternative source of pathogen-free HSA for therapeutic applications.⁶

rHSA has been produced in a variety of hosts, including bacteria,⁷ yeast,⁸ transgenic plants,⁹ and transgenic animals.¹⁰ The first approved rHSA for clinical use was Medway, produced by Mitsubishi Tanabe Pharmaceutical Co. However, it was withdrawn from the market in 2009. In 2005, another rHSA from Novozymes Inc. branded Recombumin was approved for use as pharmaceutical excipients. Both rHSA were produced by *Saccharomyces cerevisiae* containing an expression cassette with HSA cDNA.¹¹ In China, a pharmaceutical excipient-grade rHSA from North China Pharmaceutical Company Ltd, expressed by *Pichia pastoris*, was approved by Chinese State Food and Drug Administration in 2011.¹² In April of 2020, the phase I clinical study of *Oryza sativa* rHSA (OsrHSA), a recombinant HSA derived from rice grains, was completed in the clinical trial center of West Coast Clinical Trials (WCTT) global in United States. OsrHSA is the world's first plant-derived rHSA injection produced by Wuhan Healthgen Biotechnology Corp.

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The results of phase I clinical trial showed that OsrHSA was safe and well-tolerated.¹³

Novel biotechnologies have allowed the expression of recombinant proteins (RPs) in the mammary glands of transgenic animals, among which two of the products have been approved for use in the clinic. In 2009, ATryn (antithrombin α) from GTC Biotherapeutics became the first recombinant pharmaceutical approved by Food and Drug Administration (FDA), which was expressed in the milk of transgenic goats.¹⁴ The second was a C1 esterase inhibitor from Pharming Group N.V. It is expressed in the milk of transgenic rabbits¹⁵ and was approved by European Medicines Agency in 2010 and FDA in 2014. With the above achievements, the expression of rHSA in the milk of transgenic livestock becomes an attractive way to obtain a safe, easily scalable, and cost-effective source of clinically required HSA. To achieve specific expression of rHSA in mammary gland, promoters and regulatory regions from proteins that are specifically expressed in the mammary gland during lactation are usually introduced.¹⁶ Promoters of sheep β -lactoglobulin (β -LG),¹⁷ goat β -LG,^{18,19} and mouse whey acidic protein (mWAP)^{20,21} have been reported to guide the expression of rHSA in the milk of transgenic mice and the expression level of rHSA varies from 0.001 to 11.95 g/L.

In this study, we used bovine α_{s1} -casein promoter to express rHSA in the milk of transgenic mice. We aimed to investigate the ability of bovine α_{s1} -casein promoter to guide rHSA expression in mammary gland and expand the potential for large-scale, cost-effective manufacture of rHSA in transgenic animals.

Materials and Methods

Animals

BALB/c mice were purchased from SLAC Laboratory Animal Co., Ltd (Shanghai, China) and were housed in specific-pathogen free barrier facilities. All animal experiments were approved by the Animal Ethical Committee at Shanghai Institute of Pharmaceutical Industry, which was conformed to the National Institutes of Health Guidelines on Laboratory Research and Guide for the Care and Use of Laboratory Animals (8th edition, 2011).

Construction of the rHSA Expression Vector

An HSA expression cassette was constructed as shown in **Fig. 1**. HSA minigene (cDNA of HSA with its intron 1) was synthesized according to the GenBank (NCBI accession number: AF542069). The HSA minigene was infused into the

Clal-Sall digested plasmid 807 (kindly provided by Pharming Group N.V., which contains a $2 \times$ chicken β -globin insulator, a bovine α_{s1} -casein promoter, and α_{s1} -casein 3' flank sequences) by an In-Fusion HD Cloning System (Takara, Beijing, China). The primers used for vector construction are listed in **Table 1**.

Generation and Identification of Transgenic Mice

The 23-kb HSA expression cassette including HSA minigene, $2 \times$ chicken β -globin insulator, a bovine α_{s1} -casein promoter, and α_{s1} -casein 3' flank sequences were cleaved from the expression vector via *NotI* digestion. The purified DNA fragments were subsequently microinjected into pronucleus of zygotes derived from BALB/c mice according to standard protocols. Genomic DNA was extracted from tails of 7-day-old mice for transgene screening. Primers used in a multiplex polymerase chain reaction (PCR) system (Takara, Beijing, China) are listed in **Table 1**. The reaction was processed under the following conditions: 94°C for 1 minute for pre-denaturation, 94°C for 30 seconds, 59°C for 30 seconds, and 72°C for 1 minute for 30 cycles, followed by a final extension at 72°C for 5 minutes. The transgene copy number in each transgenic mouse was determined following the protocols described in our previous report.²²

rHSA-Ectopic Transcription Analysis by RT-PCR

Total RNA was isolated with TRIzol (Invitrogen, China) from mammary gland, liver, thymus, colon, kidney, stomach, uterus, lung, and heart of transgenic mice on day 8 of lactation according to the manufacturer's instructions. PrimeScript II RTase (Takara, Beijing, China) and oligo-dT primer were used to synthesize cDNA from 10 μ g RNA. PCR was then performed using HSA primer pairs HRT-5 and HRT-3 and actin primer pairs Actin-5 and Actin-3 (**Table 1**), resulting in a 160 bp and a 263 bp products, respectively.

Detection of rHSA Expression in Milk Samples

Milk samples of both nontransgenic and transgenic mice were collected on the 8th day of lactation after parturition.²³ The milk sample was diluted 1:500 in phosphate-buffered saline (PBS) and defatted by centrifugation at 1,600 g for 20 minutes. Then 10 μ L diluted milk samples were loaded on the SDS-PAGE (sodium dodecyl-sulfate polyacrylamide gel electrophoresis) for Western blotting analysis. Milk from nontransgenic mice was used as the negative control while the HSA standard (Sigma, United States) was the positive control. After protein transfer, the 0.45 μ m PVDF membrane (Millipore, United States) was

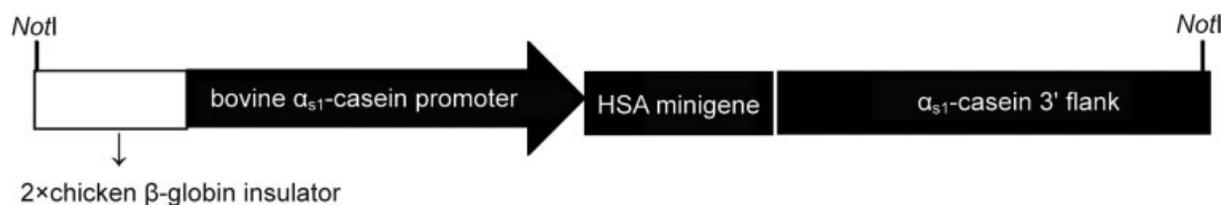


Fig. 1 Schematic structure of HSA minigene expression cassette. The expression cassette contains 2.4 kb $2 \times$ chicken β -globin insulator, 6.3 kb α_{s1} -casein promoter, 2.3 kb HSA minigene, and 7.9 kb α_{s1} -casein 3' flank sequences. The arrows indicate the direction of the transcription. HSA, human serum albumin.

Table 1 Oligonucleotide primers used in this study

Primer name	Oligonucleotide sequences (5'→3')	Products
8Hmini-5	TGATCATCAACCCATCGATGCCACCATGAAGTGGGTAACCT	2,539 bp HSAmi gene full length for vector construction
8Hmini-3	GTCCTCAGAATTCGTCGACTTATAAGCCTAAGGCAGCTTGAC	
HMP-5	TCTTTAGCTCGGCTTATTCC	1,300 bp fragment of HSA minigene for multiple PCR
HMP-3	GTTTCATCGAGCTTTGGCAAC	
CAS450-5	TGGTGGATACACTCCTTTGCAT	450 bp casein promoter fragment for multiple PCR
CAS450-3	CTGTTCAAGGCTGTCCACTTTGA	
Actin-5	GAGACCTCAACACCCACG	263 bp actin fragment for multiple PCR
Actin-3	ATGTCACGCACGATTCCC3	
HRT-5	TGTGAAGTCTTACAAGTTATC	160 bp fragment of HSA minigene for RT-PCR
HRT-3	TCTTCAACCCTATTCTGTGA	

Abbreviations: HSA, human serum albumin; PCR, polymerase chain reaction.

blocked for 1 hour at room temperature (r.t.) in blocking buffer (TBS containing 0.2% Tween-20 and 5% nonfat milk powder). Monoclonal mouse anti-HSA antibody (Proteintech, China) diluted 1:1,000 in blocking buffer was used as the primary antibody and was incubated with the membrane for 1 hour at r.t. The membrane was then incubated with horseradish peroxidase (HRP)-conjugated goat anti-mouse antibody (Beyotime, Shanghai, China) (diluted at 1:1,000 in blocking buffer) for 1 hour at r.t. Electrochemiluminescence western blotting substrate (ThermoScientific, United States) was used for detection.

Enzyme-Linked Immunosorbent Assay for HSA Concentration Determination

The concentration of HSA in the milk of transgenic mice was determined by a human albumin ELISA (enzyme-linked immunosorbent assay) kit (RayBio, United States) according to the user's manual.

Results

Generation of HSA Transgenic Mice

The rHSA expression cassette was cleaved from the vector by *NotI* digestion. About 15 ng of such fragment was microinjected into 359 fertilized BALB/c mouse eggs which were then reimplanted into pseudopregnant female mice ($n=8$). The microinjection was stopped until the size of fertilized mouse eggs was enlarged by approximately 1.5 times. Female mice ($n=6$) were pregnant and gave birth to 28 mice, 8 of which were transgenic positive as determined by multiplex PCR. ▶ **Fig. 2** shows that there were three PCR products obtained from DNA samples of the eight founders, namely a 263-bp fragment of actin, a 450-bp fragment of bovine α_{s1} -casein promoter, and a 1,300-bp fragment of HSAmi gene. However, in nontransgenic and wild-type (WT) samples, only the 263-bp fragment of actin was amplified.

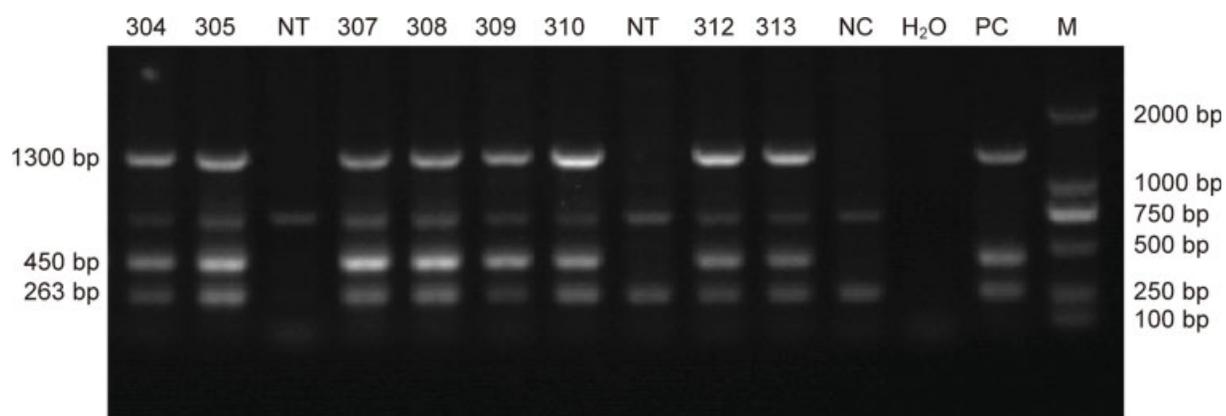


Fig. 2 Identification of transgenic mice by multiplex PCR. DNA extracted from 7-day-old mouse tail was used as the template for multiplex PCR. Mixtures of three pairs of primers (shown in ▶ **Table 1**) were added to the multiplex PCR reaction system. Positive samples were recognized to be able to produce a 1,300 bp HSA minigene fragment, a 450 bp fragment of bovine α_{s1} -casein promoter, and a 263 bp fragment of actin (indicated in the left). NT: non-transgenic mice. 304, 305, 307, 308, 309, 310, 312, 313: transgenic mice. H₂O: H₂O as the template. NC: negative control, genome DNA extracted from wild-type mouse. PC: positive control-plasmid containing the HSA minigene expression cassette as a template. M: marker DL2000 (Takara, Beijing, China). HSA, human serum albumin; PCR, polymerase chain reaction.

Table 2 The copy number of the F0 founders

F0 founders	304	305	307	308	309	310	312	313
Transgene copy number	8	12	5	1	2	7	2	1

The copy number of the eight F0 founders was detected by absolute quantitative PCR with the 3D Digital PCR technology, with the results shown in ▶Table 2. Four founders with relatively high copy number were bred with WT BALB/c mice to yield F1 generations. Multiplex PCR and copy number determination were conducted for both F0 and F1 generations. Positive female F1 transgenic mice (304–10, 305–12, 307–1, 308–1, 309–8, 310–5, 312–2, 313–10) containing the same copy number with the F0 founders were selected to be impregnated for the milk collection.

rHSA Transcription Specificity in Different Tissues

rHSA transcription specificity was analyzed by reverse transcription PCR (RT-PCR) from nine different tissues including mammary gland, stomach, lung, heart, liver, thymus, colon, kidney, and uterus. Tissues were isolated from an F1 female transgenic mouse 305–12 on the 10th day of lactation. As shown in ▶Fig. 3, strong transcription of rHSA was found in the mammary gland with no HSA transcription in other eight tissues. Similar results were obtained in F1 female transgenic mice from other four lines (304, 307, 310, 312; data not shown).

The Expression of rHSA in the Milk of Transgenic Mice

Milk was collected from eight positive female F1 transgenic mice (304–10, 305–12, 307–1, 308–1, 309–8, 310–5, 312–2, 313–10) on the 8th day of lactation. The F1 transgenic mice selected for milk analysis contained the same copy number with its corresponding F0 founders. Milk from a female WT BALB/c mouse was used as the negative control and HSA

standard as a positive control. In addition, 15 μ L milk samples with a dilution of 1:500 in PBS and 50 ng standard HSA were loaded on the SDS-PAGE for Western blotting analysis. After incubation with a monoclonal HRP-conjugated goat anti-mouse antibody, a band with a molecular weight of \sim 66 kDa, the size of HSA standard, was observed in all positive transgenic mouse milk samples, indicating an expression of rHSA in the mammary gland of transgenic mice. No band was found in the milk sample from the WT BALB/c mouse (▶Fig. 4). No significant signals were detected in transgenic lines 308 and 309, and a very weak signal in 313.

Since no signal was detected in transgenic F1 from transgenic lines 308 and 309, and a weak signal in 313 by Western blotting, the expression level of rHSA in the milk of transgenic F1 female mice from transgenic lines 304, 305, 307, 310, 312, and female WT mice was measured by ELISA according to the manufacturer's instructions. The concentration of rHSA from different transgenic mice is listed in ▶Table 3. The highest level detected was 1.98 ± 0.12 g/L in the transgenic line of 304.

Discussion

To meet the growing demand for HSA in the clinic, many efforts have been made in past decades to express rHSA in different hosts as alternatives of plasma-derived HSA. rHSA has been successfully expressed in bacteria,⁷ yeast,⁸ plants,⁹ and animals.¹⁰ The earliest host to be used to produce rHSA was *Escherichia coli* which is currently the most common expression system for a great number of RPs.²⁴ Most rHSA expressed in *E. coli* was insoluble and in the form of inclusion body, which may be due to aggregation of overexpressed protein and lack of proper folding in the cytoplasm.²⁵ Sharma and Chaudhuri⁷ have optimized the cell growth conditions during protein production in *E. coli* and obtained an enhanced (\sim 60%) expression of soluble rHSA and an increased activity compared with the original process condition. Various species including *S. cerevisiae*,^{26–28} *Hansenula polymorpha*,^{29–31} *Kluyveromyces lactis*,^{32–34} and

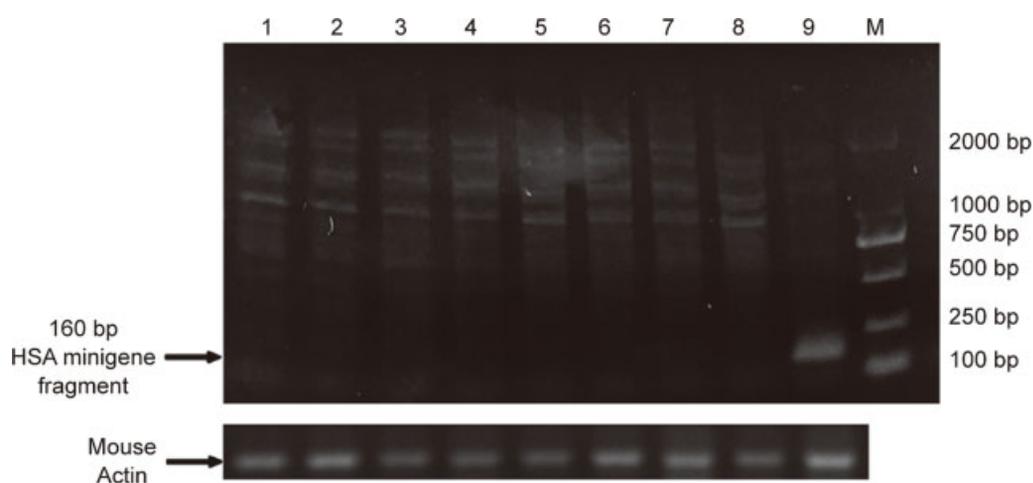


Fig. 3 rHSA transcription specificity in mammary gland. cDNA of different tissues was synthesized after RNA was extracted from liver, thymus, colon, kidney, stomach, uterus, lung, heart, and mammary gland (lanes 1–9) of the F1 transgenic female mouse on day 10 of lactation. HRT-5 and HRT-3 primers were used in RT-PCR to detect a product of 160 bp which was clearly and specifically found in cDNA of mammary gland. A 263 bp actin fragment was amplified as an internal control. rHSA, recombinant human serum albumin.

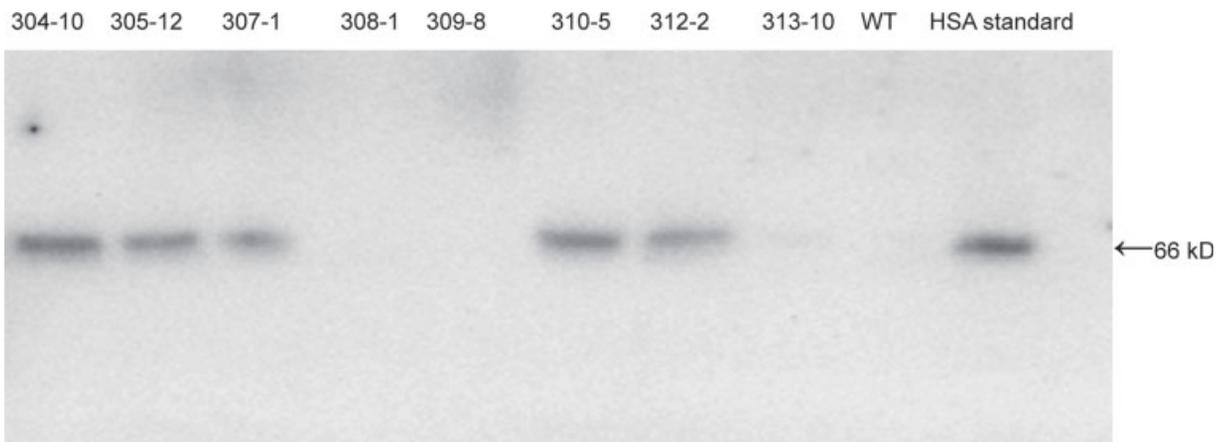


Fig. 4 Detection of rHSA expression in transgenic mice by Western blotting. The skimmed milk samples from different mice were diluted at 1:500 in PBS and separated on a 10% SDS-PAGE. Lanes 304–313: milk samples of transgenic F1 generation from different transgenic mouse lines. WT: milk from WT mice. A monoclonal mouse-anti-human albumin was used as the first antibody diluting at 1:1,000. rHSA, recombinant human serum albumin; WT, wild type.

Table 3 Concentration of rHSA in the milk of different transgenic mice

F0 founders	F1 female mice	rHSA expression level in milk (g/L)
304	304–10	1.98 ± 0.12
305	305–12	1.53 ± 0.11
307	307–1	1.49 ± 0.08
310	310–5	1.75 ± 0.11
312	312–2	1.32 ± 0.08

Abbreviation: rHSA, recombinant human serum albumin.

Note: Milk was collected from F1 transgenic females on the 8th day of lactation and defatted before the test. ELISA test was conducted following the protocols in the kit (Catalog #: ELH-Albumin, RayBio, United States). Each sample was tested in triplicate.

P. pastoris^{35–37} were constructed to express rHSA for industrial use. With the alcohol oxidase 2 (AOX2) promoter, a strong and strictly regulated promoter in yeast, *P. pastoris* became more attractive for industrial rHSA production.³⁸ In our previous study, the expression level of rHSA in *P. pastoris* reached up to 8.8 g/L in fermentation after optimization.⁸ Transgenic plants offer another way for rHSA expression, including tobacco leaves,³⁹ potato tuber,⁴⁰ and rice seed.⁴¹ He et al demonstrated a large-scale production of OsrHSA with a purity >99% and a productivity rate of 2.75 g/kg brown rice.⁴² The level of OsrHSA reached 10.58% of the total soluble protein of the rice grain. Recently, Sedaghati et al illustrated the transient expression of HSA gene in tobacco leaves by agroinfiltration, which highly depends on tobacco genotypes, *Agrobacterium* strains used for cotransfection, and harvesting time.⁹ With the ability to ensure correct posttranslational modifications for RPs, the mammary gland of transgenic animals has become a promising alternative bioreactor for the production of RPs, including rHSA. rHSA was successfully expressed in the milk of mice with expression levels vary from 0.002 to 11.9 g/L.⁶ Wu et al have constructed a 37-kb mWAP-genomic HSA hybrid gene locus and obtained transgenic mice which expressed rHSA at an extremely high level of 11.9 g/L.²¹ Larger animals with higher production of milk

such as goat⁴³ and cattle¹⁹ were also used for rHSA production. Echelard et al have used a somatic cell nuclear transfer (SCNT) technique and developed a herd of transgenic cattle among which rHSAs express in the levels from 1 to 2 g/L.¹⁹

Typically, to express a RP in the milk of a transgenic animal, an expression vector containing a long 5'-region (1–7 kb) with a promoter, enhancers that increase the expression of proteins in mammary glands, and a gene sequence encoding the targeted protein, is constructed.⁴⁴ These promoters and regulatory regions come from genes coding for proteins that are specifically expressed in the mammary gland during lactation.⁴⁴ Due to conservation between the transcriptional factors which regulate the production of milk proteins in mammary glands, promoters from one animal species usually can mediate effective transcription of the transgene in the mammary gland of another animal species.⁴⁵ Until recently, various regulatory sequences of mammary gland-specific genes, such as WAP-promoter, α_{s1} -casein promoter, β -casein promoter, and β -lactoglobulin (β -LG) promoter, have been isolated and verified in transgenic animals.⁴⁶ Since bovine α_{s1} -casein is the most abundant protein in bovine milk, the bovine α_{s1} -casein promoter has been widely used as a mammary-specific regulator to express a variety of RPs such as lactoferrin,⁴⁷ lysozyme,⁴⁸ and granulocyte colony-stimulating factor.⁴⁹ For rHSA expression, mWAP and β -LG from sheep and goat were the most frequently used promoters in mice. However, approximately 50% of the transgenic lines controlled by the WAP and β -LG promoters failed to produce detectable levels of rHSA in the milk.^{20,50} Other regulatory elements such as insulators and 3'-untranslated region of a gene, which may contribute to mRNA transcription and stability, are also used in transgenic animals.⁵¹ The chicken β -globin insulator (HS4 insulator) with a length of 1200 bp acts as a universal transcription regulator which can increase the activity of weak promoters.⁵² It has been reported that the HS4 insulator significantly increased the expression level of targeted protein in different transgenic animals.^{53–56}

The widely used technique for the generation of transgenic animals needs microinjection of a transgene-containing DNA fragment into the pronucleus of a fertilized oocyte, which is then transplanted into a pseudopregnant mother. During this

period, the transgene-containing DNA fragment is randomly integrated into the recipient genome via natural processes of genomic DNA breakage and repair.⁵⁷ Therefore, the randomness of transgene integration and the uncontrolled variability in the number of transgene copies lead to a harboring selection of a large number of primary transgenic animals. Nowadays, several new technologies enabling transgenes to be integrated into a specific genomic site have been explored to obtain a transgenic line with effective production of targeted protein. New technologies among them are the SCNT technology,¹⁹ site-specific recombinases system, and site-specific nucleases system such as zinc finger nuclease,⁵⁸ and transcription activator-like effector nuclease.⁵⁹ With the rapid development of targeted genome editing technologies such as the CRISPR/Cas9 system,⁶⁰ exogenous transgene expression in the milk and the use of transgenic animals as bioreactors have become increasingly more important and promising. It will make the transgenesis process more efficient and reduce the cost of the production of RPs. Besides the use of these new technologies, selection of different elements in the expression cassette is also an alternative way to improve rHSA expression in transgenic animals, including the selection of different promoters and the use of HS4 insulator, which acts as a barrier and protects a gene against chromosomal position effects.⁶¹

In this study we have demonstrated the feasibility of using bovine α_{s1} -casein promoter to induce the expression of human rHSA in transgenic mice. Therefore, the regulatory elements for controlling HSA expression in transgenic animals were expanded beyond those already reported promoters such as mWAP²⁰ and β -LG from sheep and goat.⁵⁰ Concerning protein expression in transgenic animals, the required quantity of the protein and the reproductive capacity of the animals should be considered for the selection of transgenic animals.⁶² Transgenic mice are a good choice to test expression constructs prior to the generation of larger founder transgenic animals because of a shorter period of reproductive maturity, less time taken between generations, and more numbers of offspring.⁶³ Meanwhile, existence of adverse effects on the health of animals caused by expressed heterogeneous protein can be determined by transgenic mice in a relatively inexpensive and rapid manner. However, the small amount of milk derived from transgenic mice limited the expression of RPs to milligram levels.⁶⁴ Here, we used transgenic mice as a model to illustrate the expression of human rHSA under the control of bovine α_{s1} -casein promoter. Our further aim is to express human rHSA for production using this regulatory element in larger transgenic animals. Our effort helps to provide a convincing evidence for this promoter using in rHSA production in larger transgenic animals such as rabbits, sheep, and cows, which have much higher production of milk than mice.

In this study we have constructed an rHSA expression cassette which contained cDNA and intron 1 of HSA gene under the control of the bovine α_{s1} -casein promoter. The involvement of introns in a transgene is generally considered as a way of increasing the transgene expression level.⁶⁵ In some cases, the use of a minigene increases the transgene expression level in comparison with the use of cDNA. This also reduces the overall size of the genetic construct

compared with a full-length transgene copy.¹⁷ Therefore, in this study we synthesized an HSA minigene which contains the cDNA of HSA and an intron 1. A 2 × chicken-globin insulator was added upstream of the promoter as a transcription enhancer, while a 7.9 kb α_{s1} -casein 3' flank sequence was used to strengthen the transcription termination located at the downstream of the expression cassette. Since the bovine α_{s1} -casein promoter has been used to express several proteins in different transgenic animals besides mice, here we chose transgenic mice as a model system to demonstrate the expression of rHSA using the bovine α_{s1} -casein promoter as the regulatory element. The addition of HS4 insulator to the expression cassette is another strategy for improving HSA expression. As shown in the result, rHSA was successfully detected in the milk of transgenic-positive mice. RT-PCR showed rHSA expression only in the mammary gland, indicating the tissue specificity of the bovine α_{s1} -casein promoter in directing transgene transcription in transgenic mice. The highest expression level of rHSA detected by ELISA was 1.98 ± 0.12 g/L, comparable with the rHSA expression level reported. Although such expression level is not the highest among those reported in transgenic mice, a higher rHSA level is possible to achieve through more selection of transgenic mice. No and very weak signals were detected in three lines with a low copy number. The highest expression level of rHSA was detected in a line with transgene copy number of 7 but not the highest copy number of 12, and in this study, we found that the difference in expression level among transgenic mice was less obvious than that was detected in copy numbers. Such results suggested that a high copy number may not be a necessary factor for a high expression level of the RPs in transgenic mice. Other factors such as integration sites of foreign fragments, the interaction between foreign fragments and genome DNA, and the interaction between regulatory elements in the expression cassette and the regulatory elements in mice genome may also affect the expression level of rHSA in transgenic mice. Overall, our results suggest the ability of the bovine α_{s1} -casein promoter to induce successful expression of rHSA in the mammary gland of transgenic mice.

Conclusion

In conclusion, we have obtained transgenic mice that produce rHSA in the milk under the control of the bovine α_{s1} -casein promoter. The rHSA expression is tissue-specific, which only occurs in the mammary gland. Our strategy of rHSA expression construct provides an alternative of successful expressions of rHSA, and probably other RPs, in the mammary glands of transgenic mice.

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Conflict of Interest

All authors declare no conflict of interest.

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