Genetics and Molecular Research

Methylation analysis of *CXCR1* in mammary gland tissue of cows with mastitis induced by *Staphylococcus aureus*

Y.J. Mao[1,2], X.R. Zhu[1], R. Li[1], D. Chen[1], S.Y. Xin[1], Y.H. Zhu[1], X.X. Liao[1], X.L. Wang[1], H.M. Zhang[1], Z.P. Yang[1] and L.G. Yang[2]

[1]Key Laboratory for Animal Genetics, Breeding, Reproduction and Molecular Design of Jiangsu Province, College of Animal Science and Technology, Yangzhou University, Yangzhou, China
[2]College of Animal Science and Technology, Huazhong Agricultural University, Wuhan, China

Corresponding author: L.G. Yang
E-mail: yangliguo2006@qq.com

Genet. Mol. Res. 14 (4): 12606-12615 (2015)
Received February 4, 2015
Accepted May 15, 2015
Published October 19, 2015
DOI http://dx.doi.org/10.4238/2015.October.19.4

ABSTRACT. Mastitis is the most important disease in the global dairy industry, and causes large economic losses. *Staphylococcus aureus* is one of most common pathogens that cause bovine mastitis. *CXCR1* has been implicated as a prospective genetic marker for mastitis resistance in dairy cows; *CXCR1* expression significantly increases when cows have mastitis. To investigate the mechanisms involved in its increased expression, bisulfite sequencing polymerase chain reaction (PCR) was used to detect the methylation status of *CXCR1* CpG island, and quantitative fluorescence PCR was used to detect *CXCR1* expression in bovine mammary tissue induced with *S. aureus* in three Chinese Holstein cows. No CpG island was found for bovine *CXCR1* in the upstream 2-kb region, whereas one CpG island that contained 13 CpG sites was found in exon 1 of *CXCR1*. All of the CpG sites were under hypermethylation from 90 to 100% in the mammary tissues. When the

mammary gland mRNA expression of *CXCR1* was 12.10-fold higher in infected cow quarters than in uninfected quarters, the methylation levels of the CpG site at position 519 were significantly lower in the infected quarters than in the uninfected quarters. Pearson correlation analysis showed that the methylation level at position 519 was significantly negatively correlated with the *CXCR1* mRNA expression level ($P < 0.05$). These results indicate that the methylation of the CpG site at position 519 may regulate *CXCR1* expression in cows with mastitis induced by *S. aureus*, but further studies are needed to elucidate the mechanisms involved.

Key words: Cow; *CXCR1*; Methylation; BSP; Mastitis; *Staphylococcus aureus*

INTRODUCTION

Mastitis is the most important disease in the global dairy industry, and causes large economic losses (Nash et al., 2003). The worldwide incidence rate of mastitis is reported to be 25-60% (Ruegg, 2003), and China has an even higher rate. Once cows are infected with a pathogen, the number of somatic cells in the mammary glands increases rapidly. Most studies have found that cows with somatic cell counts (SCCs) of less than 1×10^5 cells per milliliter in milk are not likely to be infected with the major mastitis-causing pathogens, while cows with SCCs greater than 5×10^5 are probably infected (Halasa et al., 2009). However, SCC is an inflammatory endpoint and varies between lactations, as well as in response to various environmental factors. Therefore, the development of effective molecular markers for mastitis resistance in dairy cattle is necessary.

Staphylococcus aureus is a Gram-positive and contagious bacterium that is one of the most prevalent causes of mastitis in both humans and cattle (Barkema et al., 2006). Although infection can result in obvious clinical mastitis, *S. aureus* often evades the immune system, resulting in a sub-clinical chronic infection that can persist for the life of the animal (Sutra and Poutrel, 1994). Cytokines are an important group of inflammatory mediators. Proinflammatory cytokines rapidly promote inflammation after the perception of the pathogen, and anti-inflammatory cytokines suppress and confine the activity of proinflammatory cytokines. *CXCR1* codes for a major proinflammatory cytokine (Dinarello, 2000), and has been implicated as a prospective genetic marker for mastitis resistance in dairy cows. Bovine *CXCR1*, located on autosome 2 at 90.3 cM in *Bos taurus*, contains a 2219-bp intron and a 1803-bp exon (Pighetti et al., 2012). *CXCR1* is expressed on the surface of neutrophils and is essential for neutrophil migration to the mammary glands, and for the resolution of bacterial infections (Del Rio et al., 2001; Proudfoot, 2002). Bovine *CXCR1* is highly polymorphic, and as many as 36 single-nucleotide polymorphisms (SNPs) have been identified in the coding region and surrounding sequences of *CXCR1* in Holstein dairy cows (Pighetti et al., 2012). Several SNPs in bovine *CXCR1* have been implicated in resistance to mastitis C in several sample populations of Holstein dairy cows (Youngerman et al., 2004; Leyva-Baca et al., 2008; Goertz et al., 2009; Chen et al., 2011; Pighetti et al., 2012; Zhou et al., 2013).

DNA methylation is one of the key epigenetic modifications (Weber et al., 2007) that stably decreases gene expression and plays a crucial role in inflammatory disease (Vanselow et

al., 2006). The potential prognostic value of promoter hypermethylation of the α_{s1}-*casein* gene has been demonstrated in mammary gland tissues of dairy cows with acute mastitis (Vanselow et al., 2006). Recently, an association between the methylation patterns of the *CD4* promoter in dairy cow peripheral blood cells and mastitis was reported (Wang et al., 2013); however, the DNA methylation patterns of the bovine *CXCR1* gene and their relationship with mastitis status is still unclear.

In the present study, the DNA methylation pattern of bovine *CXCR1* was determined in the mammary gland tissues of Chinese Holstein cows infected with *S. aureus*. *CXCR1* expression was also measured using quantitative reverse transcription polymerase chain reaction (qRT-PCR). The objective of this study was to investigate the correlation between *CXCR1* CpG island methylation status and mRNA expression, in order to provide a theoretical basis for resistance to mastitis infection in dairy cows.

MATERIAL AND METHODS

Experimental animals and sampling

All of the procedures involving animals received approval from the Institutional Animal Care and Use Committee of Yangzhou University. Three primiparous Holstein cows in mid-lactation were used for this study. At the beginning of the study, these cows had been clinically healthy for over two consecutive weeks, based on a composite milk SCC of less than 200,000 cells/mL, and had no clinical signs of mastitis or any other disease during early lactation. All of the quarters from these cows were bacteriologically tested using milk samples to confirm that they were not sub-clinically infected with an invading pathogen. The cows were housed and fed in individual tie-stalls, had free access to water, and were milked twice daily at 6:00 am and 6:00 pm. The cows averaged 19.2 ± 4.4 kg milk/day and were 142 ± 25 days in milk at the start of the trial.

An *S. aureus* strain (strain ATCC29213) was obtained from the China General Microbiological Culture Collection Center and grown aerobically overnight on tryptic soy agar in a humidified incubator at 37°C. A single colony of *S. aureus* was transferred to a 50-mL conical tube containing 20 mL of tryptic soy broth and incubated at 37°C in an open-air shaker at 225 rpm. The bacteria were grown until an OD600 nm of 0.6 was reached, when they were plated in triplicate on their respective media to confirm the number of bacteria per milliliter. The broth culture was diluted in sterile Ringer's solution to yield 5,000,000 CFU in a 5-mL volume inoculum. Following afternoon milking, the inoculum was infused into one rear quarter of each cow via a sterile disposable syringe fitted with a sterile teat cannula by the full insertion infusion method, and one front control quarter of each cow was infused with 5 mL ice-cold pyrogen-free phosphate-buffered saline (pH 7.4). Prior to inoculation, the challenged teats were rigorously cleaned with cotton balls containing 70% alcohol. Immediately following inoculation, all of the teats were immersed in a post-milking teat disinfectant containing 1% iodine with lanolin. Systemic and local inflammatory indicators were used to monitor the clinical response to the intramammary infection (IMI) challenge. Rectal temperature, respiration rate, and udder appearance were evaluated at 0, 6, 12, and 24 h post-challenge. Based on preliminary experiments, the peak clinical signs of *S. aureus* inoculation were expected about 24 h post-inoculation. At 24 h post-inoculation and before the peak clinical signs, the mammary gland tissues of the *S. aureus*-infected and uninfected quarters were sampled in a surgical operation performed by an experienced veterinarian. Duplicate samples of quarter foremilk

were aseptically collected for bacteriological examination and SCC immediately before the challenge, and 12 and 24 h post-challenge. The SCC was determined using infrared procedures (SCC-100, ChemoMetec, Denmark). Bovine mammary gland tissues were immediately stored in liquid nitrogen until further use.

Bioinformatic analysis

The analysis and identification of CpG islands in a 3083-bp fragment of *CXCR1* (GenBank accession No. NM_001105038.1, GJ060584), including the upstream 2-kb region and exon, was performed using Methyl Primer Express® version 1.0 (http://www.urogene.org/methprimer/index1.html).

Methylation analysis

Genomic DNA was extracted from bovine mammary gland tissue by standard phenol/chloroform extraction, and subjected to bisulfite conversion using an EpiTect bisulfite kit (Qiagen, Valencia, CA, USA) according to the manufacturer instructions. PCR was used to amplify the bisulfite-treated DNA. The primers were designed using Primer Premier 5.0 (http://www.premierbiosoft.com/primerdesign/index.html). The following sequences were selected for use as primers: F, 5'-TGGGTCAAGTTCATATGTTTAGGC-3'; R, 5'-CCATGTGATTGCTGAGACCTGT-3'. The 20-μL reactions contained 1 μL DNA template, 2 μL 10X PCR buffer, 1 μL 10 μM/L forward primer, 1 μL 10 μM/L reverse primer, 1.6 μL 10 mM dNTPs, 0.4 μL 5 U/μL *Taq* polymerase, and 13 μL water. PCR amplification was performed according to the following protocol: 95°C for 1 min and 30 cycles at 94°C for 30 s, 50°C for 30 s, and 72°C for 1 min followed by 72°C for 5 min. The PCR products were subjected to electrophoresis on 1% agarose gels using a DL2000 (TaKaRa, Japan) as a reference standard, and excised, purified, and inserted into a pMD18-T vector (TaKaRa). Recombinant clones were used to transform *Escherichia coli* TB1 cells. Positive recombinant clones were selected on lysogeny broth agar plates containing 100 μg/mL ampicillin and confirmed by PCR and DNA sequencing (about 20 positive recombinant clones were selected from each individual).

Real-time PCR analysis

RNA was isolated from mammary tissue using TRIzol reagent (Invitrogen, Carlsbad, CA, USA) according to the manufacturer instructions. The RNA yield was determined using a NanoDrop 2000 spectrophotometer (Thermo Scientific, USA), and the integrity was evaluated using agarose gel electrophoresis stained with ethidium bromide. Quantification was performed in a two-step reaction process: reverse transcription (RT) and PCR. Each RT reaction contained 0.5 μg RNA, 2 μL PrimeScript buffer, 0.5 μL oligo dT, 0.5 μL random 6mers, and 0.5 μL PrimeScript RT Enzyme Mix I (TaKaRa), in a total volume of 10 μL. The reactions were performed in a GeneAmp® PCR System 9700 (Applied Biosystems, USA) for 15 min at 37°C, followed by heat inactivation of RT for 5 s at 85°C. The 10-μL RT reaction mix was then diluted ten times in nuclease-free water and stored at -20°C. Real-time PCR was performed using a LightCycler® 480 II real-time PCR instrument (Roche, Switzerland) in a 10-μL PCR reaction mixture that contained 1 μL cDNA, 5 μL 2X LightCycler® 480 SYBR® Green I Master (Roche), 0.2 μL forward primer, 0.2 μL reverse primer, and 3.6 μL nuclease-free water. The

reactions were incubated on a 384-well optical plate (Roche) at 95°C for 10 min, followed by 40 cycles at 95°C for 10 s and at 60°C for 30 s. The *CXCR1* fragment was amplified using the primers listed in Table 1, and *β-ACTB* primers were used as an internal control; the expression of *CXCR1* in each sample was normalized to that of *β-ACTB*. Triplicate PCR amplifications were performed for each sample.

Table 1. Reverse transcription polymerase chain reaction primers.

Primer	Primer sequence	Length (bp)
CXCR1	5'-GAGGACCTGGGTGCCAATA-3' 5'-GGTGAATCCGTAGCAGAAC-3'	111
β-ACTB	5'-GGATGCAGAAAGAGATCACT-3' 5'-TCTGCTGGAAGGTGGACA-3'	129

Data processing and analysis

Methylation sequencing results were processed by the QUMA analysis software (http://quma.cdb.riken.jp), and the real-time PCR results were processed using the $2^{-\Delta\Delta Ct}$ method (ΔCt = mean *CXCR1* expression - mean *β-ACTB* expression) (Shaw et al., 2007). Data are reported as means ± SD. A Student *t*-test was used to compare the different groups in SPSS 17.0 (SPSS Inc., Chicago, IL, USA), and the methylation and mRNA expression levels were analyzed by Pearson correlations. Differences were considered to be statistically significant at $P < 0.05$.

RESULTS

Indicators of clinical response to the IMI challenge

All of the cows developed both local and systemic responses to the IMI challenge and developed mastitis. The infected quarters exhibited signs of inflammation, such as redness, swelling, tenderness, and hardness, and flaky, watery, or yellow-colored mammary secretions were observed in the milk after inoculation (i.e., between 11-24 h post-inoculation). The milk SCC in the challenged quarters (3,400,000 cells/mL) was significantly higher 20 h after the IMI challenge than at 0 h after the IMI challenge (180,000 cells/mL; $P < 0.01$). A significant increase in *S. aureus* growth was observed in the inoculated quarters ($P < 0.01$).

Bioinformatic analysis

The results of the Methyl Primer analysis showed that no CpG island was found in the bovine *CXCR1* gene upstream 2-kb region, but one CpG island was found in the exon (Figure 1). Therefore, the primers were designed to amplify a fragment that contained the whole CpG island.

Validation of the CpG island fragment amplification

The products of the bisulfite PCR primer pair amplification from the DNA extracted from the mammary gland were examined by electrophoresis on 1% agarose gel. The sizes of the amplified fragments corresponded with the expected PCR product sizes (378 bp), and each amplified a single specific product that could be directly cloned and sequenced (Figure 2).

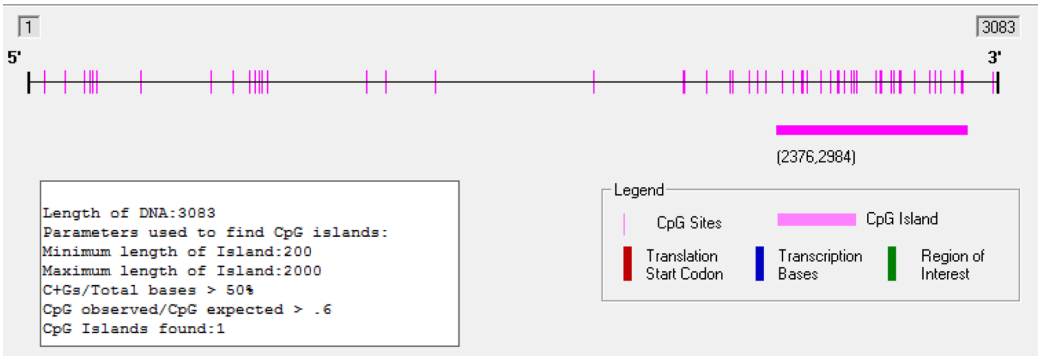

Figure 1. Bioinformatic analysis of a CpG island of bovine CXCR1, including the upstream 2-kb region and exon.

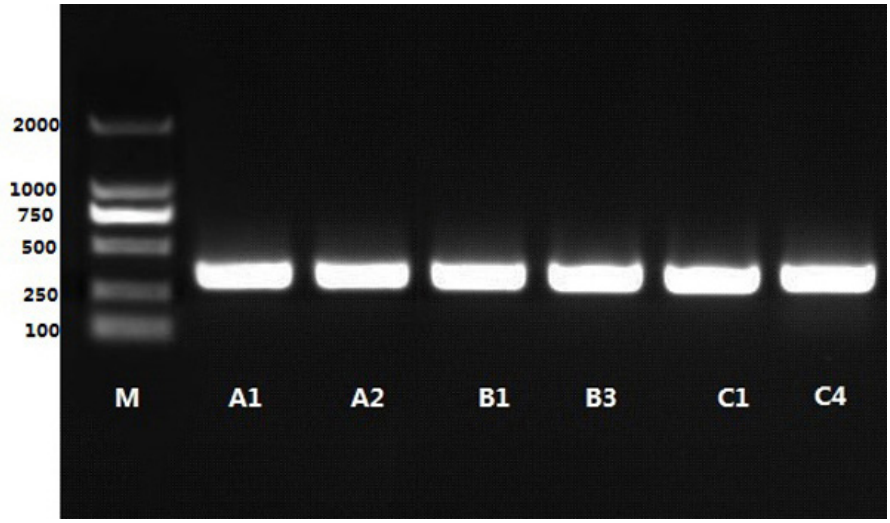

Figure 2. Agarose gel (1%) electrophoresis for *CXCR1* polymerase chain reaction products. A1, B1, and C1 were uninfected cow quarters; A2, B3, and C4 were infected cow quarters. *Lane M*, DL2000 molecular weight markers.

Methylation level analysis

A total of 126 correct clones of the CpG island were obtained and confirmed by sequencing, and the average number of recombinant clones for each individual was 21.0 (range 20-22). All of the CpG sites were methylated (Figure 3). Overall, the CpG island methylation levels for the infected and uninfected quarters were 0.96 ± 0.01 and 0.97 ± 0.01, respectively. Methylation levels were not significantly different between the infected and uninfected quarters for each CpG site or for the overall CpG island methylation levels, except for CpG1 at position +519 of the exon ($P > 0.05$). The methylation levels were 100% for the CpGs at +614, +622, and +706 in the uninfected and infected quarters, and these sites were excluded from the correlation analysis.

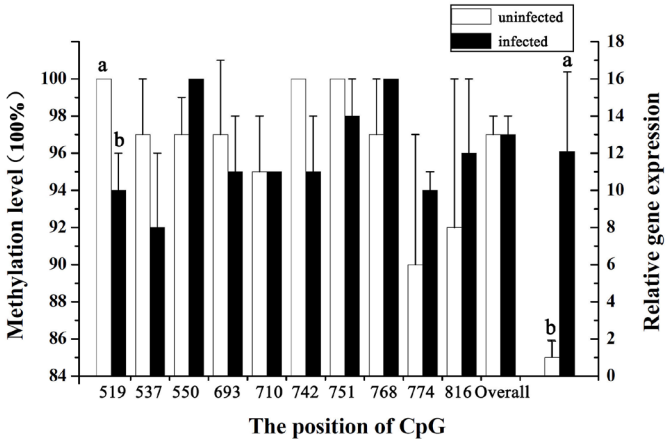

Figure 3. Average methylation levels of overall and single CpG sites in the bovine *CXCR1* gene in infected and uninfected cow quarters (means ± SD). Means with different superscripts within the same column significantly differed ($P < 0.05$). The far-right column represents the relative gene expression of *CXCR1* in infected and uninfected quarters.

Correlation between methylation level and mRNA expression

The *CXCR*1 mRNA relative expression levels in the infected quarters were significantly higher than in the uninfected quarters (12.10 ± 4.28 *vs* 1.00 ± 0.09; $P < 0.05$). The Pearson correlation analysis revealed that methylation status was negatively correlated with *CXCR*1 mRNA relative expression level for most of the CpG islands (6/13), with significant correlation coefficients for CpG1 at position +519 (Figure 4).

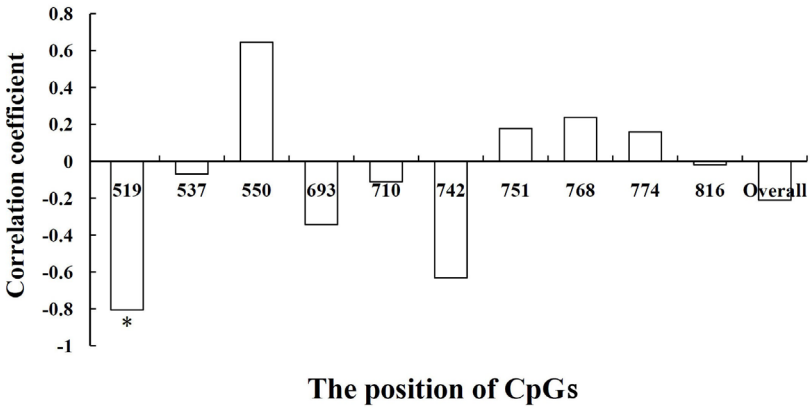

Figure 4. Correlation analysis of methylation levels and mRNA expression at different CpG sites of bovine *CXCR1*. The x-axis indicates the positions of CpG sites, and the y-axis indicates the correlation coefficients between the methylation level of individual CpG sites and the expression of *CXCR1* (Pearson correlation). *Significant at $P < 0.05$.

DISCUSSION

DNA methylation at promoter CpG sites leads to the repression of gene expression by altering the conformation of DNA and local histone structures (Ng and Bird, 1999; Cedar and Bergman, 2009). In our study, no CpG island was found in bovine *CXCR1* upstream 2-kb region, but one CpG island was found in the exon. Multiple alignments revealed the amplifieds region located in the position from 481 to 858 bp in the coding region of *CXCR1*. Several studies on gene methylation related to cow mastitis have been conducted. Vanselow et al. (2006) reported that the promoter region of DNA for the gene a_{S1}*-casein* is hypomethylated in the bovine lactating mammary gland. During *E. coli* infection of the mammary gland, this region becomes methylated at three CpG dinucleotides. These changes accompany the shutdown of a_{S1}*-casein* synthesis, with mRNA levels decreasing to 50% and protein levels to 2.5% of those in non-mastitic control glands. Similar results have been obtained for a *Streptococcus uberis* infection of the mammary gland. Methylation levels at three CpG sites for the a_{S1}*-casein* are much higher in mastitic tissue (ranging from 28 to 68%) than in non-mastitic tissue (ranging from 10 to 25%) (Swanson et al., 2009; Singh et al., 2010). Wang et al. (2013) reported that the bovine *CD4* promoter had 16% more methyl groups in cows with clinical mastitis (75.0 ± 5.8%) than in healthy cows (59.0 ± 8.5%). DNA methylation in the coding region is generally an important silencer (Hsieh, 1997; Irvine et al., 2002), and recent evidence suggests that methylation in the coding region silences human gene expression (Zhu et al., 2005). However, the demethylation of CpG sites in intra- and/or extra-genic positions has been shown to increase the transcription rate for a specific gene (Klose and Bird, 2006). In our study, all of the CpG sites were highly methylated, and the methylation level of CpG1 at position +519 in infected cow quarters was significantly lower than that in uninfected quarters. *CXCR1* expression in infected quarters was significantly higher than in uninfected quarters. CpG1 demethylation may increase gene expression, and *CXCR1* could be regarded as an important genetic or epigenetic marker in bovine mastitis induced by *S. aureus*.

At present, there are several alternative hypotheses for the association between intragenic DNA methylation and transcription, including modifying transcription efficiency, altering the local histone conformation, and producing different levels of sense and antisense mRNA. Interestingly, recent evidence suggests that variable methylation levels of individual CpG sites affect the binding affinity of transcription factors to nearby binding sites (Flower et al., 2010; Rishi et al., 2010). This may suggest an alternative mechanism by which specific intragenic CpG sites can affect transcription. In human breast cancer, the expression of *ESR1* is of significant biological and prognostic importance, and it largely drives the clinical management plan. *ESR1*-positive tumors have broadly better outcomes, with slower-growing tumors, fewer metastases, and a greater range of hormonally targeted treatment options, including tamoxifen and aromatase inhibitors. Methylation data of *ESR1* show a typical pattern of methylation, with an unmethylated promoter and a highly methylated intragenic region, and the methylation level is above 0.9 for the CpGs in the exons (Li et al., 2010; Shenker and Flanagan, 2012). The methylation characteristics of *ESR1* are similar to those of *CXCR1*. Considering the importance of *CXCR1* in cow mastitis, future studies should examine the epigenetic regulation of this gene using larger sample sizes.

CONCLUSIONS

We identified 13 CpG sites in the exon region of *CXCR1* from the mammary tissue of Chinese Holstein cows challenged with *S. aureus*. We suggest that CpG1 at position +519 is a critical site in the regulation of *CXCR1* expression. These results indicate that the methylation of CpG1 may regulate the expression of *CXCR1* in cow mastitis induced by *S. aureus*, but the mechanisms involved need to be elucidated.

Conflicts of interest

The authors declare no conflict of interest.

ACKNOWLEDGMENTS

Research supported by the National Natural Science Funds (#31272407 and #31372286), the China Postdoctoral Science Foundation (#20110491169), the Priority Academic Program Development of Jiangsu Higher Education Institutions (PAPD), and the New Century Talent Plan of Yangzhou University.

REFERENCES

Barkema HW, Schukken YH and Zadoks RN (2006). Invited review: the role of cow, pathogen, and treatment regimen in the therapeutic success of bovine *Staphylococcus aureus* mastitis. *J. Dairy Sci*. 89: 1877-1895.

Cedar H and Bergman Y (2009). Linking DNA methylation and histone modification: patterns and paradigms. *Nat. Rev. Genet*. 10: 295-304.

Chen RJ, Yang ZP, Ji DJ, Mao YJ, et al. (2011). SNPs of *CXCR1* gene and its associations with somatic cell score in Chinese Holstein cattle. *Anim. Biotechnol*. 22: 133-142.

Del Rio L, Bennouna S, Salinas J and Denkers EY (2001). CXCR2 deficiency confers impaired neutrophil recruitment and increased susceptibility during *Toxoplasma gondii* infection. *J. Immunol*. 167: 6503-6509.

Dinarello CA (2000). Proinflammatory cytokines. *Chest* 118: 503-508.

Flower K, Hellen E, Newport MJ, Jones S, et al. (2010). Evaluation of a prediction protocol to identify potential targets of epigenetic reprogramming by the cancer associated Epstein Barr virus. *PLoS One* 5: e9443.

Goertz I, Baes C, Weimann C and Reinsch N (2009). Association between single nucleotide polymorphisms in the *CXCR1* gene and somatic cell score in Holstein dairy cattle. *J. Dairy Sci*. 92: 4018-4022.

Halasa T, Nielen M, De Roos AP and Van HR (2009). Production loss due to new subclinical mastitis in Dutch dairy cows estimated with a test-day model. *J. Dairy Sci*. 92: 599-606.

Hsieh CL (1997). Stability of patch methylation and its impact in regions of transcriptional initiation and elongation. *Mol. Cell Biol*. 17: 5897-5904.

Irvine RA, Lin IG and Hsieh CL (2002). DNA methylation has a local effect on transcription and histone acetylation, *Mol. Cell Biol.* 22: 6689-6696.

Klose RJ and Bird AP (2006). Genomic DNA methylation: the mark and its mediators. *Trends Biochem. Sci.* 31: 89-97.

Leyva-Baca I, Schenkel F, Martin J and Karrow NA (2008). Polymorphisms in the 5' upstream region of the *CXCR1* chemokine receptor gene, and their association with somatic cell score in Holstein cattle in Canada. *J. Dairy Sci.* 91: 407-417.

Li Y, Zhu J, Tian G, Li N, et al. (2010). The DNA methylome of human peripheral blood mononuclear cells. *PLoS Biol.* 8: e1000533.

Nash DL, Rogers GW, Cooper JB, Hargrove G, et al. (2003). Heritability of intramammary infections at first parturition relationships with sire transmitting abilities for somatic cell score, udder type traits, productive life, and protein yield. *J. Dairy Sci.* 86: 2684-2695.

Ng HH and Bird A (1999). DNA methylation and chromatin modification. *Curr. Opin. Genet. Dev*. 9: 158-163.

Pighetti GM, Kojimaa CJ, Wojakiewicza L and Rambeaud M (2012). The bovine *CXCR1* gene is highly polymorphic. *Vet. Immunol. Immunopathol*. 145: 464-470.

Proudfoot AE (2002). Chemokine receptors: multifaceted therapeutic targets. *Nat. Rev. Immunol.* 2: 106-115.

Rishi V, Bhattacharya P, Chatterjee R, Rozenberg J, et al. (2010). CpG methylation of half-CRE sequences creates C/EBPalpha binding sites that activate some tissue-specific genes. *Proc. Natl. Acad. Sci. U. S. A.* 107: 20311-20316.

Ruegg PL (2003). Investigation of mastitis problems on farms. *Vet. Clin. North Am. Food Anim. Pract*. 19: 47-73.

Shaw AE, Reid SM, Ebert K, Hutchings GH, et al. (2007). Implementation of a one-step real-time RT-PCR protocol for diagnosis of foot-and-mouth disease. *Virol. Methods* 143: 81-85.

Shenker N and Flanagan JM (2012). Intragenic DNA methylation: implications of this epigenetic mechanism for cancer research. *Br. J. Cancer* 106: 248-253.

Singh K, Molenaar AJ, Swanson KM and Stelwagen K (2010). DNA methylation is associated with a suppression of aS1-casein gene expression during involution and infection of the bovine mammary gland. IDF World Dairy Summit, Auckland.

Sutra L and Poutrel B (1994). Virulence factors involved in the pathogenesis of bovine intramammary infections due to *Staphylococcus aureus*. *J. Med. Microbiol.* 40: 79-89.

Swanson KM, Stelwagen K, Dobson J, Henderson HV, et al. (2009). Transcriptome profiling of *Streptococcus uberis*-induced mastitis reveals fundamental differences between immune gene expression in the mammary gland and in a primary cell culture model. *J. Dairy Sci*. 92: 117-129.

Vanselow J, Yang W, Herrmann J and Zerbe H (2006). DNA-remethylation around a STAT5-binding enhancer in the alphaS1-casein promoter is associated with abrupt shutdown of alphaS1-casein synthesis during acute mastitis. *J. Mol. Endocrinol.* 37: 463-477.

Wang XS, Zhang Y, He YH, Ma PP, et al. (2013). Aberrant promoter methylation of the CD4 gene in peripheral blood cells of mastitic dairy cows. *Genet. Mol. Res.* 12: 6228-6239.

Weber M, Hellmann I, Stadler MB and Ramos L (2007). Distribution, silencing potential and evolutionary impact of promoter DNA methylation in the human genome. *Nat. Genet.* 39: 457-466.

Youngerman SM, Saxton AM, Oliver SP and Pighetti GM (2004). Association of CXCR2 polymorphisms with subclinical and clinical mastitis in dairy cattle. *J. Dairy Sci*. 87: 2442-2448.

Zhou L, Wang HM, Ju ZH, Zhang Y, et al. (2013). Association of novel single nucleotide polymorphisms of the *CXCR1* gene with the milk performance traits of Chinese native cattle. *Genet. Mol. Res.* 12: 2725-2739.

Zhu S, Goldschmidt-Clermont PJ and Dong C (2005). Inactivation of monocarboxylate transporter MCT3 by DNA methylation in atherosclerosis. *Circulation* 112: 1353-1361.

