Long noncoding RNA MALAT1 regulates sepsis in patients with burns by modulating miR-214 with TLR5

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Abstract. The present study aimed to identify the involvement of the dysregulation of the metastasis-associated lung adenocarcinoma transcript-1 (MALAT1)/microRNA (miR)-214/Toll-like receptor (TLR)5 signaling pathway in the development of post-burn sepsis. THP-1 cells were used in the present study, in addition to 8-10 week-old mice. ELISA analysis was performed to examine the expression levels of inflammation-associated factors. Reverse transcription-quantitative polymerase chain reaction (RT-qPCR) analysis and western blotting were performed to analyze the influence of burns or burns with infection on the production of MALAT1, miR-214 and TLR5. Commonly-used software and a luciferase assay was used to confirm the target gene of miR-214. RT-qPCR analysis and western blotting were performed to elucidate the effects of lipopolysaccharide (LPS), miR-214 and MALAT1 on the expression of miR-214, TLR5, tumor necrosis factor (TNF)-α, interleukin (IL)-6 and IL-10. Burn injury increased TLR5, TNF-α, IL-6 and IL-10 expression levels, which were abolished by treatment with MALAT1. miR-214 directly targeted TLR5 by binding to the TLR5 3’ untranslated region (ITR), and the luciferase activity of the wild-type, and not the mutant, TLR5 3’UTR was reduced following transfection with miR-214. In cells not treated with LPS, MALAT1 and anti-miR-214 significantly enhanced TLR5, TNF-α, IL-6 and IL-10 expression, and repressed miR-214 production; whereas, miR-214 and MALAT1 short hairpin (sh)RNA decreased TLR5, TNF-α, IL-6 and IL-10 expression levels, and increased miR-214 expression. In cells treated with LPS, LPS reduced miR-214 expression and increased TLR5, TNF-α, IL-6 and IL-10 expression compared with LPS-untreated cells, and the effects of MALAT1, anti-miR-214, miR-214 and MALAT1 shRNA on TLR5, TNF-α, IL-6 and IL-10 were the same as in LPS-untreated cell. The results of the present study indicated the association between the dysregulation of MALAT1/miR-214/TLR5 and the risk of post-burn sepsis.

Introduction

Serious burn trauma is destructive damage associated with a hyper-metabolic and catabolic state. Furthermore, it compromises the immune system by activating systemic inflammation, frequently leading to suboptimal innate immunity (1). Damage to the skin barrier and alterations in immunity elevate the susceptibility of patients with burns to infections in the burn wound, and may cause systemic bacterial spread, triggering multi-organ failure, sepsis and mortality (1). A variety of patient factors, including depth of burn, extent of injury, immune status and age, combined with microbial factors, including toxin production, enzymes, number and type of organisms and mobility, are determinants of the possibility of aggressive burn wound infection. Burns with a large surface area are associated with infections in the burn wound leading to multi-organ failure, sepsis and mortality (2,3). Infection is a frequent trigger of complications in patients with serious burns. Burns trigger damage to the barrier that protects the skin. This provides an optimal opportunity for microbial colonization and likely spread into the blood and other organs, which may cause sepsis. Therefore, infection is the principal factor leading to mortality in patients with burns who survive the early phase of burn shock (4). Williams et al (5) performed a clinical observational study and demonstrated that sepsis accounted for 47% of the mortality in patients with burns at a large pediatric burn center. The study of Mann et al (6) reported that patients with burns have a high incidence of sepsis and associated unfavorable outcomes.

The majority of transcribed RNAs are non-coding in mammalian cells (7). Numerous RNAs produce small RNAs, including microRNAs (miRs), a well-known family of small RNAs, in addition to small nucleolar RNAs, tRNA-derived stress-induced RNAs, transcription initiation RNAs, Piwi-interacting RNAs and small interfering RNAs (8). Other transcripts [long non-coding RNAs (IncRNAs)] consist of >200 nucleotides (9). ncRNAs impact the modulation of genes at all levels via their interactions with proteins, RNA and DNA, including protein stability, mRNA translation, mRNA turnover, pre-mRNA splicing, transcription and chromatin remodeling (10).
A previous study demonstrated that metastasis-associated lung adenocarcinoma transcript-1 (MALAT1) has a regulatory role in the generation of inflammatory cytokines in certain human cancers, including lung, liver, breast, cervical and bladder cancers (11). Yan et al (12) first demonstrated the alteration in MALAT1 expression in the retina in patients with diabetes. It has been demonstrated that the glucose-mediated upregulation of the inflammatory mediators tumor necrosis factor (TNF)-α and interleukin (IL)-6 is modulated by lncRNA MALAT1 via stimulation of serum amyloid A3 (SAA3); it was identified that early elevation, followed by a reduction in MALAT1, positively increases the expression of inflammatory ligands, including SAA3, finally enhancing the expression of inflammatory cytokines, including TNF-α and IL-6 (13). Further duration-dependent studies at different phases of diabetes using animal models (long-term, short-term and acute) may indicate whether this lncRNA, MALAT1, is an actual initiator of oxidative stress and inflammation (13). The flagellin receptor Toll-like receptor 5 (TLR5) is among the most frequently-triggered pattern recognition receptors (14). Allelic variations in TLR5 may make infants prone to sepsis, and high-level production of TLR5 in septic individuals is positively associated with more serious disease (14,15). Using computational analysis, TLR5 was identified to be a potential target gene of miR-214 in the present study, and TLR5 has been reported to be functionally involved in the pathogenesis of post-burn sepsis; the occurrence of sepsis is important for the prognosis of patients with burns (16,17). The present study analyzed the association between the dysregulation of MALAT1, miR-214 or TLR5 and the risk of post-burn sepsis.

**Materials and methods**

**Mouse model of burn injury and burn wound infection.** The Institutional Animal Care and Use Committee of Baoji Center Hospital (Baoji, China) approved all procedures involving animals. A total of 48 female C57BL/6 mice at 8-10 weeks of age (weight, 21.2±2.8 g) were obtained from the Animal Center of Baoji Center Hospital and used in the present study. Mice were housed in the appropriate institutional animal care facility, and acclimatized for 7 days post-arrival at 25°C with 60% humidity. The acclimatization was performed under a 12/12 light/dark cycle; mice were provided food and water ad libitum. A well-established mouse model with full-thickness cutaneous burn injury was utilized in the present study. Buprenorphine (0.1 mg/kg) was utilized to treat the mice subcutaneously for 30 min for analgesia prior to the burn injury. Isoflurane (2.5%) was used for general anesthesia. The dorsal surface of the mouse was shaved, and 1 ml normal saline was used to treat the mice subcutaneously to avoid injury to the underlying tissues. A 35% total surface area scald burn was caused by exposing the shaved dorsal area to 97-98°C water for 10 sec using a molded template with a rectangular opening. A total of 2 ml Ringer's lactate solution (Kelun Pharmaceutical, Chengdu, China) was utilized to treat the mice immediately for fluid resuscitation via the intraperitoneal route, and buprenorphine (0.1 mg/kg) was used to treat the mice secondarily post-burn procedure. Burn-injured mice were housed in sterile cages individually, and allowed food and sterile water. Burn wounds were collected for analysis under anesthesia. *Pseudomonas aeruginosa* bacteria were seeded on the surface of the burn wound. A total of 1x10^6 colony-forming units/ml (200 µl) *Pseudomonas aeruginosa* were incubated on the surface of the burn wound 4 days post-burn to cause wound infection. A hematology analyzer was used to determine blood cell count of the animal groups.

**Cell culture and transfection.** THP-1 cells were obtained from the Chinese Cell Bank of the Chinese Academy of Sciences (Shanghai, China). RPMI medium (Gibco; Thermo Fisher Scientific, Inc., Waltham, MA, USA) containing 10% heat-inactivated fetal bovine serum (Life Technologies; Thermo Fisher Scientific, Inc.,), 100 µg/ml streptomycin, 50 U/ml penicillin and 2 mM glucose (Euroclone Diagnostica S.r.l., Siziano, Italy) was used to maintain the THP-1 cells, in a humidified incubator with 5% CO₂ at 37°C. In order to perform cell transfections, THP-1 cells were cultured in 24-well plates at a concentration of 2x10^5 cells per well, and Attractene Transfection Reagent (Qiagen GmbH, Hilden, Germany; cat. no. 1051531) was used to transfect 15 pmol/ml miR-675-5p mimic (Life Technologies; Thermo Fisher Scientific, Inc.; cat. no. 4464066) or miR-675-5p inhibitor (Life Technologies; Thermo Fisher Scientific, Inc.; cat. no. 4464084) or scramble control (miR-NC 5′-CTA AGGTTAAGTCGCCCTCGCT-3′) or 15 pmol/ml MALAT1 shRNA into THP-1 cells. Each reaction was performed in triplicate; analysis was conducted 48 h following transfection.

**THP-1 cell polarization and lipopolysaccharide (LPS) treatment.** A total of 20 ng/ml interferon (IFN)-γ (PeproTech, Inc., Rocky Hill, NJ, USA) with 10 ng/ml LPS (Sigma-Aldrich, St. Louis, MO) was used to direct PMA-differentiated THP-1 cells to the M1 phenotype, and 20 ng/ml IL-4 (PeproTech, Inc.) and 20 ng/ml IL-13 (Sigma-Aldrich; Merck KGaA, Darmstadt, Germany) was used to direct PMA-differentiated THP-1 cells to the M2 phenotype. THP-1 cells were seeded into 96-well plates at a concentration of 2x10^5 cells per well, and exposure to polarizing conditions was performed. Reverse transcription-quantitative polymerase chain reaction (RT-qPCR) analysis was performed to identify the polarized phenotypes. A total of 1 µg/ml LPS was used to treat THP-1 cells for 24 h when the cells had grown to 80% confluence.

**RNA isolation and RT-qPCR.** TRIzol reagent (RNAprep Pure Cell/Bacteria kit; Tiangen Biotech Co., Ltd., Beijing, China) was used to isolate the total RNA from THP-1 cells and peripheral blood mononuclear cells (PBMCs) from blood samples collected from the experimental mice, in accordance with the manufacturer's protocol. A PrimeScript™ RT Reagent kit (Perfect Real-Time; Takara Bio, Inc., Otsu, Japan) was used to perform reverse transcription to obtain cDNA according to the manufacturer's protocols, using 2 µg cell-extracted RNA or 300 ng total tissue RNA from the burn wound as the template. LightCycler 480 SYBR Green I Master (Roche, Basel, Switzerland) was used to perform qPCR analysis to determine the relative expression of IncRNA MALAT1, miR-214 and TLR5. The qPCR cycling conditions was as follows: Initial polymerase activation at 95°C for 10 min, followed by 40 cycles for 15 sec at 95°C and 60 sec at 60°C. The expression of TLR5 mRNA (F: 5′-CTTTCTTGGGGCGCAATAAAC-3′;
The present study established animal models of burn injury and post-burn infection, and it was identified that burn injury caused a significant decrease in the white blood cell count compared with the sham controls (Fig. 1A) using a hematology analyzer. In addition, post-burn infection caused a further decrease in white blood cell count compared with the sham and burn groups, which was eliminated by treatment with short hairpin (sh)RNA-MALAT1 (shMALAT1) (Fig. 1B). Burn injury caused a significant increase in TNF-α (Fig. 1B), IL-6 (Fig. 1C) and IL-10 (Fig. 1D), and post-burn infection caused a further increase in TNF-α (Fig. 1B), IL-6 (Fig. 1C) and IL-10 (Fig. 1D) compared with the burn groups, which was abrogated by treatment with shMALAT1, indicating that the burn injury and post-burn sepsis-induced inflammatory reaction may be attenuated by shMALAT1.

**MALAT1, miR-214 and TLR5 are associated with burn injury and post-burn sepsis.** An evident increase in MALAT1 mRNA (Fig. 2A) expression levels was observed in the burn group.
Figure 1. Elevation of proinflammatory cytokines caused by burn injury or post-burn infection is attenuated by treatment with MALAT1. A mouse model of burn injury and burn wound infection was established, peripheral blood samples were collected from all mice and monocytes were isolated from the blood samples. WBC count, TNF-α, IL-6 and IL-10 were detected. Burn injury induced a decrease in (A) WBC count, and post-burn infection caused a greater decrease in WBC count, which was abolished following transfection with shMALAT1. Burn injury induced an increase in (B) TNF-α, (C) IL-6 and (D) IL-10, and post-burn infection caused a greater increase in TNF-α, IL-6 and IL-10 expression, which was abolished by transfection with shMALAT1. *P<0.01 vs. sham. Veh, vehicle; sh, short hairpin; TNF-α, tumor necrosis factor-α; IL, interleukin; MALAT1, metastasis-associated lung adenocarcinoma transcript-1; WBC, white blood cell.

Figure 2. MALAT1, miR-214 and TLR5 are associated with burn injury and post-burn sepsis. Peripheral blood samples were collected from all mice, and monocytes were isolated from the above blood samples. MALAT1, miR-214 and TLR5 were detected in the monocytes. (A) The burn and post-burn infection groups exhibited significantly upregulated MALAT1 expression. (B) Burn injury inhibited miR‑214 expression, and the inhibitory effect on miR‑214 expression was greater in the burn plus infection group; however, treatment with shMALAT1 restored miR‑214 expression. (C) Burn injury promoted TLR5 expression, and the promoting effect on TLR5 expression was greater in the burn plus infection group; however, the effect was abrogated by shMALAT1. *P<0.01 vs. sham. miR, microRNA; TLR5, Toll-like receptor 5; sh, short hairpin; MALAT1, metastasis-associated lung adenocarcinoma transcript-1.
compared with the sham control; post-burn infection caused a further increase in its expression. By contrast, a significant decrease in miR-214 expression (Fig. 2B) was observed in the burn group compared with the sham control, and post-burn infection further enhanced this decrease, which was attenuated by treatment with shMALAT1. The results for TLR5 expression (Fig. 2C) were comparable to those for MALAT1. The increase in the expression of TLR5 was attenuated by treatment with shMALAT1.

**TLR5 is a candidate target gene of miR-214.** TLR5 and TNF receptor-associated factor 3 (TRAF3; Fig. 3) were predicted to be target genes of miR-214 using online miRNA databases, including TargetScan. The putative binding sequences of miR-214 in the 3'-UTRs of TLR5 and TRAF3 are conserved among different species. To substantiate whether miR214 targets TLR5 and TRAF3 directly, luciferase reporter vectors were constructed containing a wildtype or mutant 3'-UTR of TLR5 and TRAF3, in which the sequences corresponding to the seed sequences were altered. As presented in Fig. 3B, miR-214 mimics significantly reduced the luciferase activity of wild-type TLR5 compared with the control, whereas the luciferase activity of mutant TLR5 (Fig. 3B), wild-type and mutant TRAF3 (Fig. 3D) in miR-214-overexpressing cells exhibited no significant difference, indicating that TLR5 is a virtual target gene of miR-214.

**MALAT1 and miR-214 alter the production of inflammation-associated factors.** LPS is considered to be a major toxin in sepsis, and is the primary cause of the enhanced production of pro-inflammatory cytokines that is observed in sepsis (19). The expression levels of miR-214, TLR5, TNF-α, IL-6 and IL-10 were assessed in LPS-treated or untreated THP-1 cells transfected with MALAT1, anti-miR-214, MALAT1 shRNA and miR-214. As presented in Fig. 4, transfecting with MALAT1 and anti-miR-214 reduced miR-214 expression (Fig. 4A), while enhancing TLR5 mRNA (Fig. 4B) and protein (Fig. 4D), IL-6 (Fig. 4E) and IL-10 (Fig. 4F) expression compared with the scramble control.

Cells treated with LPS exhibited a lower level of miR-214 (Fig. 5A) compared with that in untreated cells. Furthermore, MALAT1 and anti-miR-214 induced a further decrease in the miR-214 expression level compared with the scramble control. By contrast, cells treated with LPS displayed higher levels of TLR5 mRNA (Fig. 5B) and protein (Fig. 5C), TNF-α (Fig. 5D), IL-6 (Fig. 5E) and IL-10 (Fig. 5F) compared with untreated cells. Furthermore, treatment with MALAT1 and anti-miR-214 induced a decrease in TLR5 mRNA (Fig. 5B) and protein (Fig. 5C), TNF-α (Fig. 5D), IL-6 (Fig. 5E) and IL-10 (Fig. 5F) compared with the scramble control. Furthermore, the suppression effects of miR-214 were comparable to those for MALAT1. The results for TLR5, TNF-α, IL-6 and IL-10 expression were further enhanced. Finally, MALAT1 shRNA and miR-214 induced a further decrease in miR-214 expression compared with the scramble control, and the suppression effects of miR-214 on TLR5, TNF-α, IL-6 and IL-10 expression were further enhanced. LPS-treated cells exhibited a lower expression level of miR-214 (Fig. 7A) and higher expression levels of TLR5 mRNA (Fig. 7B) and protein (Fig. 7C), TNF-α (Fig. 7D), IL-6 (Fig. 7E) and IL-10 (Fig. 7F) compared with untreated cells. Furthermore, MALAT1 shRNA and miR-214 induced a further decrease in miR-214 expression compared with the scramble control, and the inhibitory effects of miR-214 on the expression levels of the above factors appeared to be increased in cells treated with
LPS compared with those in cells not treated with LPS. The results collectively suggested that MALAT1 promoted TLR5, TNF-α, IL-6 and IL-10 expression by inhibiting miR-214.

**Discussion**

The discovery of MALAT1, and other lncRNAs including p50-associated COX-2 extragenic RNA, Lethe and nuclear factor (NF)-κB interacting IncRNA, as novel NF-κB modulators, has added complexity to the understanding of the regulation of this crucial transcription factor, which is important in inflammation and immunity (20). It has been demonstrated that MALAT1 is necessary for the close control of inflammatory reactions triggered by LPS, revealing the potential role of MALAT1 in modulating inflammation and innate immunity (20). Recently, a report identified that the glucose-mediated increase in the inflammatory mediators IL-6 and TNF-α is modulated by MALAT1 via stimulation of SAA3 (13). It has been reported that MALAT1, which was initially regarded as a prognostic marker for non-small cell lung cancer, may serve a role in regulating the expression of inflammatory mediators (20). As a highly conserved long non-coding RNA, in addition to nuclear-enriched transcript 2, MALAT1 is deregulated in a range of cancer types. MALAT1 was identified as a prognostic marker for lung cancer metastasis, and has also been associated with a number of other human cancer types (11). MALAT1 functions as an oncogene in esophageal squamous cell carcinoma (ESCC), and it modulates ESCC growth by adjusting the ataxia telangiectasia mutated serine/threonine kinase -checkpoint kinase 2 pathway (21). In the present study, it was identified that burn injury decreased the white blood cell count, and the suppressive effect of post-burn infection on WBCs was much stronger, although this was eliminated by shMALAT1. Burn injury up-regulated MALAT1, TLR5, TNF-α, IL-6 and IL-10 expression levels, while the suppressive effects of post-burn infection on TNF-α, IL-6 and IL-10 expression levels were stronger, and were abrogated by shMALAT1. Furthermore,
the present study investigated whether MALAT1, miR-214 and TLR5 were associated with burn injury and post-burn sepsis, and revealed that MALAT1 promoted burn severity by inhibiting miR-214 production and further enhancing TLR5 expression.

Previous reports have demonstrated the role of miR-214 in inflammatory responses in vitro. Li et al. (22) demonstrated that miR-214 is markedly increased in human monocytes and THP-1 cells following treatment with advanced glycation end products, compromises phosphatase and tensin homolog (PTEN) expression and postpones the apoptosis of THP-1 cells. Jindra et al. (23) demonstrated that miR-214 is also markedly elevated in active T cells and is important for enhancing the proliferation and activation of T cells by affecting PTEN. It has been indicated that overexpression of miR-214 markedly enhances IL-6 and TNF-α expression in bone marrow derived macrophages (24). Gonzales et al (25) reported that miR-214 expression was substantially increased, which is consistent with our findings, in an inflammatory macrophage model. Increasing evidence demonstrates that a large portion of lncRNAs are able to function as miRNA sponges by sharing common miRNA response elements, impacting regulation by suppressing available miRNA activity at the post-transcriptional level. It has been demonstrated the rs619586A >G single-nucleotide polymorphism is able to trigger the capture of miR-214 by MALAT1 (15). It has also been revealed that the miR-214 directly targets MALAT1 with the rs619586G allele and inhibits the expression of MALAT1, and miR-214 inhibitors reverse this inhibitory effect; miR-214 inhibitors may release the

Figure 5. Treatment of LPS altered expression levels of miR-214, TLR5, TNF-α, IL-6 and IL-10 in THP-1 cells or LPS-treated cells transfected with Sh-MALAT1, Sh-anti-miR-214. Expression levels of miR-214, TLR5, TNF-α, IL-6 and IL-10 in THP-1 cells or LPS-treated cells transfected with Sh-MALAT1, Sh-anti-miR-214 were determined using reverse transcription-quantitative polymerase chain reaction, western blot and ELISA analyses. (A) Treatment with LPS decreased miR-214 expression compared with untreated cells. The miR-214 expression level in LPS-treated cells transfected with Sh-MALAT1 or Sh-anti-miR-214 were significantly decreased compared with the scramble control, and this effect was more marked with Sh-anti-miR-214. (B) LPS-treated cells displayed a higher level of TLR5 mRNA compared with untreated cells. TLR5 mRNA expression levels in LPS-treated cells transfected with Sh-MALAT1 or Sh-anti-miR-214 were significantly increased compared with the scramble control, and this effect was more marked with Sh-anti-miR-214. (C) LPS increased the TLR5 protein expression level compared with that in untreated cells. TLR5 protein expression in LPS-treated cells transfected with Sh-MALAT1 and Sh-anti-miR-214 were further increased. (D) TNF-α expression in LPS-treated cells was significantly increased compared with LPS-untreated cells, while TNF-α expression in LPS-treated cells transfected with Sh-MALAT1 and Sh-anti-miR-214 were further increased compared with LPS-treated cells. (E) LPS-treated cells exhibited a higher level of IL-6 compared with LPS-untreated cells, while IL-6 expression in LPS-treated cells transfected with Sh-MALAT1 and Sh-anti-miR-214 was further increased compared with LPS-treated cells. (F) IL-10 expression in LPS-treated cells was significantly increased compared with LPS-untreated cells, while IL-10 expression in LPS-treated cells transfected with Sh-MALAT1 and Sh-anti-miR-214 was further increased compared with LPS-treated cells.

Figure 5: A-G. Treatment of LPS altered expression levels of miR-214, TLR5, TNF-α, IL-6 and IL-10 in THP-1 cells or LPS-treated cells transfected with Sh-MALAT1, Sh-anti-miR-214. Expression levels of miR-214, TLR5, TNF-α, IL-6 and IL-10 in THP-1 cells or LPS-treated cells transfected with Sh-MALAT1, Sh-anti-miR-214 were determined using reverse transcription-quantitative polymerase chain reaction, western blot and ELISA analyses. (A) Treatment with LPS decreased miR-214 expression compared with untreated cells. The miR-214 expression level in LPS-treated cells transfected with Sh-MALAT1 or Sh-anti-miR-214 were significantly decreased compared with the scramble control, and this effect was more marked with Sh-anti-miR-214. (B) LPS-treated cells displayed a higher level of TLR5 mRNA compared with untreated cells. TLR5 mRNA expression levels in LPS-treated cells transfected with Sh-MALAT1 or Sh-anti-miR-214 were significantly increased compared with the scramble control, and this effect was more marked with Sh-anti-miR-214. (C) LPS increased the TLR5 protein expression level compared with that in untreated cells. TLR5 protein expression in LPS-treated cells transfected with Sh-MALAT1 and Sh-anti-miR-214 were further increased. (D) TNF-α expression in LPS-treated cells was significantly increased compared with LPS-untreated cells, while TNF-α expression in LPS-treated cells transfected with Sh-MALAT1 and Sh-anti-miR-214 were further increased compared with LPS-treated cells. (E) LPS-treated cells exhibited a higher level of IL-6 compared with LPS-untreated cells, while IL-6 expression in LPS-treated cells transfected with Sh-MALAT1 and Sh-anti-miR-214 was further increased compared with LPS-treated cells. (F) IL-10 expression in LPS-treated cells was significantly increased compared with LPS-untreated cells, while IL-10 expression in LPS-treated cells transfected with Sh-MALAT1 and Sh-anti-miR-214 was further increased compared with LPS-treated cells. *P<0.01 vs. respective untreated group. LPS, lipopolysaccharide; miR, microRNA; Sh, short hairpin; MALAT1, metastasis-associated lung adenocarcinoma transcript-1; TLR5, Toll-like receptor 5; TNF-α, tumor necrosis factor-α; IL, interleukin.
The inhibitory effect of endogenous miR-214 on MALAT1 with the rs619586G allele, and hence trigger increased expression of MALAT1 (15). In the present study, it was demonstrated that miR-214 directly targets the TLR5 3' UTR, and miR-214 markedly inhibited the luciferase activity of the wild-type TLR5 3' UTR and had no effect on the luciferase activity of the mutant TLR5 3' UTR.

TLR5 is present on various cell types including mast cells, macrophages, monocytes, neutrophils and epithelial cells, and is the receptor for the bacterial structural protein flagellin (26). Flagellin signaling through TLR5 depends on myeloid differentiation primary response protein 88 and interleukin-1 receptor-associated kinase 1 and subsequent stimulation of the phosphatidylinositol 3-kinase, mitogen activated protein kinase and NF-κB pathways (27,28). Similar to other TLR agonists, flagellin has been demonstrated to trigger dendritic cell maturation and stimulation, thereby enhancing lymphocyte movement to secondary lymphoid sites (29,30). Others have demonstrated that spontaneous neutrophil apoptosis is delayed by flagellin via mediation of induced myeloid leukemia cell differentiation protein Mcl-1 and suppression of caspase 3 (31). The present study detected the expression levels of miR-214, TLR5, TNF-α, IL-6 and IL-10 in cells transfected with Sh-MALAT1, Sh-anti-miR-214 and miR-214 using RT-qPCR, western blotting and ELISA, and observed that MALAT1 and anti-miR-214 inhibited miR-214 expression while enhancing TLR5, TNF-α, IL-6 and IL-10 expression in LPS-untransfected or treated cells. MALAT1 shRNA and miR-214 upregulated miR-214 expression while inhibiting TLR5, TNF-α, IL-6 and IL-10 expression in LPS-untransfected or treated cells. It was also

Figure 6. Expression levels of miR-214, TLR5, TNF-α, IL-6 and IL-10 in mouse peripheral blood mononuclear cells transfected with MALAT1 shRNA and miR-214. Expression levels of miR-214, TLR5, TNF-α, IL-6 and IL-10 in mouse peripheral blood mononuclear cells transfected with MALAT1 shRNA and miR-214 were determined using reverse transcription-quantitative polymerase chain reaction, western blot and ELISA analyses. (A) shRNA and miR-214 enhanced miR-214 expression compared with the scramble control, and the effect was strongest with miR-214. (B) TLR5 mRNA expression (lower panel) and protein expression (upper panel) in the MALAT1 shRNA or miR-214 group was increased compared with the scramble control, and the effect was strongest in the miR-214 group. (C) MALAT1 shRNA was successfully transfected into the cells (upper panel) and MALAT1 shRNA and miR-214 reduced MALAT1 mRNA expression (lower panel) compared with the scramble control; this effect was strongest with miR-214 (scale bar: 10 μm). (D) Expression of TNF-α in the MALAT1 shRNA and miR-214 treatment groups was significantly decreased compared with the scramble control, and this effect was strongest in the miR-214 group. (E) Expression of IL-6 in the miR-214 treatment group was significantly decreased compared with the scramble control, and this effect was more marked in the miR-214 group. *P<0.01 vs. respective scramble group. miR, microRNA; Sh, short hairpin; MALAT1, metastasis-associated lung adenocarcinoma transcript-1; TLR5, Toll-like receptor 5; TNF-α, tumor necrosis factor-α; IL, interleukin.
revealed that LPS caused an evident decrease in miR-214 expression, while inducing a marked increase on TLR5, TNF-α, IL-6 and IL-10 levels. Sepsis has been referred to a systemic inflammation syndrome induced by infection (32). It is currently considered that the majority of patients who succumb to sepsis have unaddressed immune inhibition septic foci (33). Complex mechanisms have been implicated in cell signaling and bacterial sensing, which are regulated during sepsis (34). Immunity to bacterial infection is induced when pattern recognition receptors on phagocytes, including TLRs, identify the pathogen-associated molecular patters produced by the infecting microorganism (35). TLR5 detects bacterial flagellin. It is produced in various cell types, including epithelial cells, dendritic cells, mastocytes and monocytes (35). For instance, in a model of spontaneous colitis, TLR5-depleted mice exhibit more severe colitis (36). The production of TLR5 was elevated among septic patients compared with healthy subjects; additionally, TLR5 production was reported to be reduced in septic patients after 7 days at follow-up (14). As for TLR production, TLR5 was elevated in neutrophils and monocytes from septic patients and TLR4 and TLR2 were reduced in neutrophils in the surviving patients at follow-up (14).
There are certain limitations to this study: i) The experiments were only performed in animals and cultured cells, thus further study on human subject is required to confirm the results; and ii) the spectrum of inflammatory cytokines has not been comprehensively studied, and thus certain cytokines, including NF-κB and its associated components, should be considered in future studies.

In conclusion, the results of the present study demonstrated the association between the dysregulation of MALAT1/miR-214/TLR5 and the risk of post-burn sepsis.

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Availability of data and materials
The datasets used and/or analyzed during the present study are available from the corresponding author on reasonable request.

Authors' contributions
FG made substantial contributions to the design of the present study, analyzed the data and reviewed the manuscript. RC collected the data and prepared the manuscript; YX made substantial contributions to the acquisition and analysis of data; QZ made substantial contributions to the collected of data and literature, and prepared the manuscript; HG made substantial contributions to the design of the present study and reviewed the literature. All authors read and approved the final manuscript.

Ethics approval and consent to participate
The Institutional Animal Care and Use Committee of Baoji Center Hospital (Baoji, China) approved all procedures involving animals.

Patient consent for publication
Not applicable.

Competing interests
The authors declare that they have no competing interests.

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